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PARASITISM OF GIBBERELLA SAUBINETII ON CORN SEEDLINGS [1]

By NORMA L. PEARSON

Formerly *Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and *Assistant in the Department of Plant Pathology, University of Wisconsin* [2]

INTRODUCTION

A seedling blight of Indian corn (*Zea mays* L.) and other cereals is produced by the fusarial stage of *Gibberella saubinetii* (Mont.) Sacc. The organism also causes, later in the season, a stalk rot and ear rot of corn and a head blight of small grains (*32, 5*).[3] The fungus exists as a saprophyte on crop refuse in the soil and is capable of becoming parasitic only when it can enter the host through wounds or when unfavorable environmental conditions have so altered the plant that it is incapable of resisting penetration (*5*). In corn seedlings dark brown, sunken lesions, varying in size, develop on the mesocotyl. The lesions appear to have originated usually at the point where a root emerges. In cases of severe blighting the entire seedling is rotted.

In open-pollinated corn the disease develops below the temperature of 20° C., while above 24° the seedlings are practically immune. Several strains of corn, differing in the degree of their resistance to the attacks of the parasite, have been developed in cooperative experiments conducted by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, the Wisconsin and Illinois Agricultural Experiment Stations, and Funk Bros. Seed Co. As reported by Dickson and Holbert (*16, p. 330*)—

> The resistant inbred lines develop free from disease at temperatures as low as 12° C.; the intermediate are susceptible at temperatures below 20° and 24° C.; and the susceptible are blighted at all temperatures at which the parasite grows.

Corn is a high-temperature plant, making its best growth at about 24° C. The cell walls of the seedlings grown at this temperature are chiefly of cellulose, and the epidermis, endodermis, and cells surrounding wounds are well suberized (*15, 16*); the reserves are largely hexose in nature. The suberized walls serve at least as a partial barrier against fungus penetration; and in case invasion is accomplished, the cellulose walls, reserve hexoses, and low nitrogen content offer little food, for the fungus has demonstrated but slight ability to use these substances as a source of carbon when grown on artificial media (*14, 16*). "Resistant lines maintain a balanced metabolism even at low temperatures" (*16, p. 330–331*).

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[2] The writer wishes to express her sincere appreciation to Prof. C. E. Allen, of the department of botany, and Prof. J. G. Dickson, of the department of plant pathology, University of Wisconsin, under whose direction this work was done, for the helpful suggestions and criticisms given throughout the progress of the study and the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 594.

The susceptible lines and the more resistant ones, when grown at the lower temperatures, present a very different condition. At these lower temperatures the cell walls are built up largely of pectinlike substances; the reserves are mainly pentosans and suberization is scanty. Cultivation of the parasite on artificial media has shown that it makes its best growth on pectinlike and other pentose-yielding substances (*16*). The lack of protective suberization around wounds produced by root emergence allows the invasion of the plant, and after the fungus has once gained entrance, sufficient readily hydrolyzable food of a pectinlike nature is available in the walls and in the reserve substances.

Chemical studies of the seedlings of the various strains of corn grown at different temperatures have led Dickson to conclude that resistance and susceptibility are strikingly correlated with the metabolic activities of the seedlings grown at these different temperatures (*14*, *15*, *16*).

In connection with the genetic and chemical study of the strains of corn produced at the different temperatures and as a part of the project of the investigation of the nature of disease resistance, it seemed advisable to undertake at this time a study of the parasitism of the corn plant by *Gibberella saubinetii* and perhaps later to expand this into a cytological study of resistance.

MATERIALS AND METHODS

Three lines of corn which had been inbred for 8 to 10 generations were used as a basis for this work.[4] Two of the strains are susceptible and one shows a fair amount of resistance to *Gibberella saubinetii*. The kernels to be used were first treated with 95 per cent alcohol and then allowed to remain for one and one-half to two hours in a 0.35 per cent solution of Uspulun.[5] At the end of that time they were placed singly in test tubes each containing 8 c. c. of 1 per cent soil-extract agar. The tubes were set into racks, covered with black paper, and kept in a 12° C. temperature chamber throughout the experiment.

Six series of inoculations were made, five with 150 seedlings each and one with 60. The first inoculations were made with a fairly heavy spore suspension of *Gibberella saubinetii*,[6] but this did not prove satisfactory, because the water spread out and evaporated before the spores had an opportunity to germinate or to establish themselves in the host tissue. Inoculation by means of small squares cut from Gibberella colonies growing on potato-dextrose agar plates was found to be more satisfactory. A drop of sterile water or of spore suspension was usually added at the time of applying the inoculum, and water only was applied at intervals afterwards.

The inoculum was applied (1) on the tip of the coleorhiza immediately after it had burst through the pericarp, (2) on the rupture produced by the emergence of the primary root, (3) on the swollen

[4] These strains were furnished by J. R. Holbert, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, from Bloomington, Ill., and consist of the following inbred lines: A–R–1–2–3–7 (resistant), A–9–5–6 (susceptible), and A–4–8–5 (susceptible).

[5] An organic-mercury seed disinfectant, manufactured in Germany.

[6] The culture of *Gibberella saubinetii* (culture No. 259) was obtained from Helen Johann, of the Division of Cereal Crops and Diseases, at Madison, Wis. The organism was originally isolated by D. Atanasoff in 1918, from Wisconsin wheat. The writer wishes to acknowledge the assistance given by Miss Johann through her knowledge of *G. saubinetii* and other forms of Fusarium.

portion of the mesocotyl before any roots emerged, (4) on the ruptures on the mesocotyl produced by root emergence, (5) on the mesocotyl at some distance from such a root rupture, and (6) on the coleoptile.

Large lesions did not develop readily on the seedlings in the test tubes, and it was concluded that lack of moisture was the limiting factor. No very satisfactory means of remedying this difficulty was discovered until the fifth series of inoculations was made. In the last two series small squares of absorbent cotton were dipped in distilled water and sterilized. These were placed in the test tubes, each being halfway between the plug and the surface of the agar. By pressing the cotton against the side of the test tube it was made to adhere, filling the tube to only one-third or one-half its diameter. More large lesions developed in these series than in the previous ones.

The inoculated portions of seedlings were fixed at intervals, and adjacent portions were plated on potato-dextrose agar. The plates bearing these portions were examined after six or seven days, and if they showed contaminations the corresponding fixed material was discarded. Seedlings were numbered serially and placed in separate fixing vials.

The material was embedded and sectioned in the usual manner. A medium chromo-acetic fixative was found to give the most satisfactory results for the study of the responses of the host cells. The formula is as follows: One per cent chromic acid, 280 c. c.; glacial acetic acid, 2 c. c.; distilled water, 120 c. c. Sections were cut from 13 μ to 15 μ in thickness and stained with Flemming's triple stain or with Heidenhain's iron-alum haematoxylin, light green being used as a counter stain. For the study of the details of penetration and certain of the changes in the cell walls, Juel's fixative was used. Sections were cut from 10 μ to 12 μ in thickness and stained with Delafield's haematoxylin and orange G.

Uninjured mesocotyls, as well as those which had been wounded by pricking with a sterile needle, also were fixed and stained.

OBSERVATIONS AND DISCUSSION

PENETRATION

Penetration of the Cortex of the Mesocotyl

The adventitious roots of the corn mesocotyl originate in the pericycle of the stele; thus it is necessary for a young root to grow out through the cortex, this growth being accompanied by the disorganization of the cells in its path. The wound produced in the mesocotyl (fig. 1, A) forms an excellent channel for fungus invasion. As the young root elongates, the walls of the cells lining the rupture appear thicker (fig. 2, C) and stain with safranine. Dickson and Holbert (*16*) have shown that these walls become more or less suberized, depending on the temperature and the strain of corn. Thus a partial, if not complete, barrier may be formed against fungal penetration.

Material fixed a few days after inoculation and sectioned at the point of root emergence shows the fungus hyphae to have grown into the rupture and from there to have made their way into the intercellular spaces of the cortex. The first cells invaded appear to be those located at the external margin of the rupture. (Fig. 3, A.)

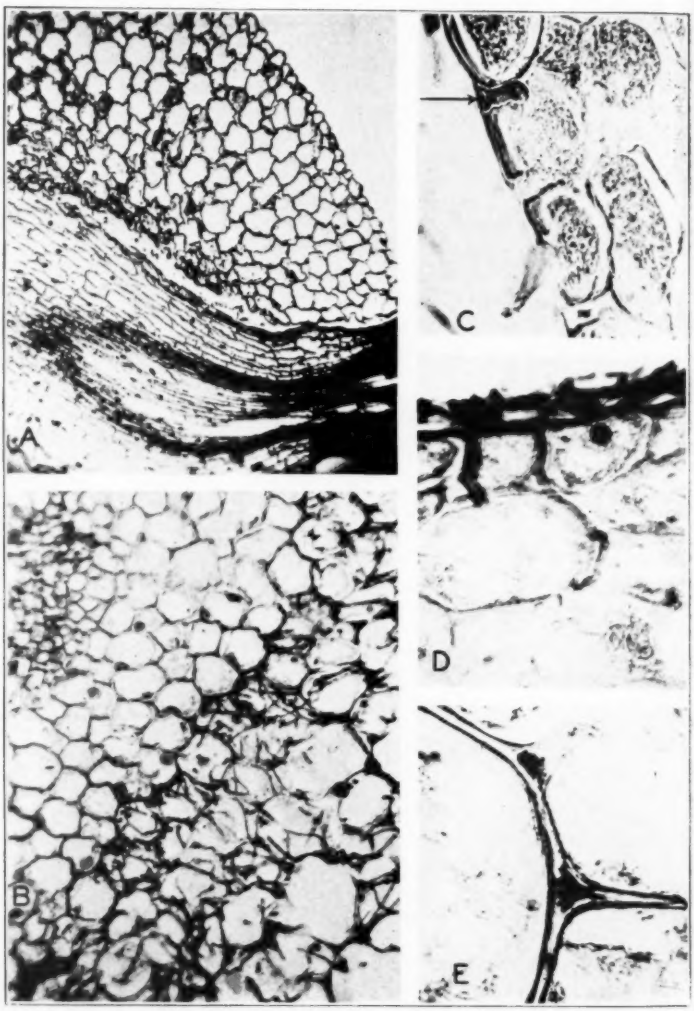

FIGURE 1.—A, Cross section of a mesocotyl showing the rupture produced in the cortex as a result of root emergence. × 60. B, Portion of an infected mesocotyl at the endodermis. (Fig. 3, *E*, *d*.) The fungus has progressed as far as the endodermis and is checked, at least temporarily. (Semi-resistant strain.) × 194. C, Portion of the epidermis of coleorhiza. (Fig. 3, D, *c*.) Arrow shows plug. × 194. D, Portion of epidermis of coleorhiza. (Fig. 3, D, *c*.) Two hyphae have penetrated between the epidermal cells at the points where plugs are formed. × 607. E, An exceedingly thickened wall from the cortex of an infected coleorhiza. × 583

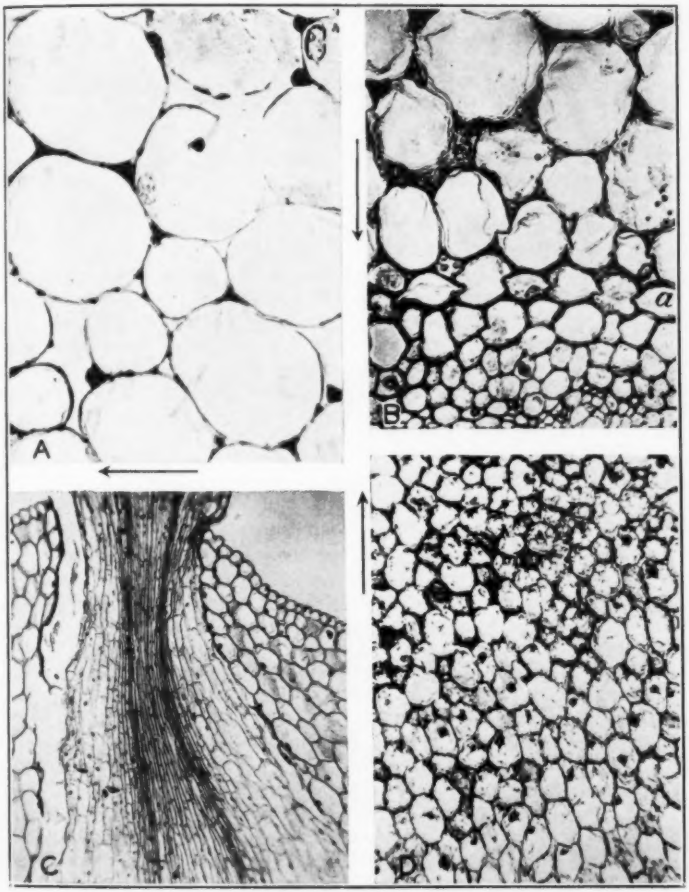

FIGURE 2.—A, Cells from a region adjacent to a large mesocotyl lesion. Stainable material has accumulated in the intercellular spaces. × 302. B, Portion of an infected mesocotyl. (Fig. 3, E.) The advance of the fungus is checked at the endodermis (*a*). (Semiresistant strain). × 235. C, A rupture in the mesocotyl produced by the emergence of a root. The walls of the cells lining the rupture are thickened and stain deeply. Nuclear orientation is seen in the cells adjacent to the rupture. × 68. D, Cortical cells from an area adjacent to a large mesocotyl lesion. The cells nearest the fungus have more conspicuous contents than have those at some distance away. The accumulation of stainable material in the intercellular spaces and the deeply staining character of the walls are also shown. × 63. Arrows indicate direction of fungus or of penetration

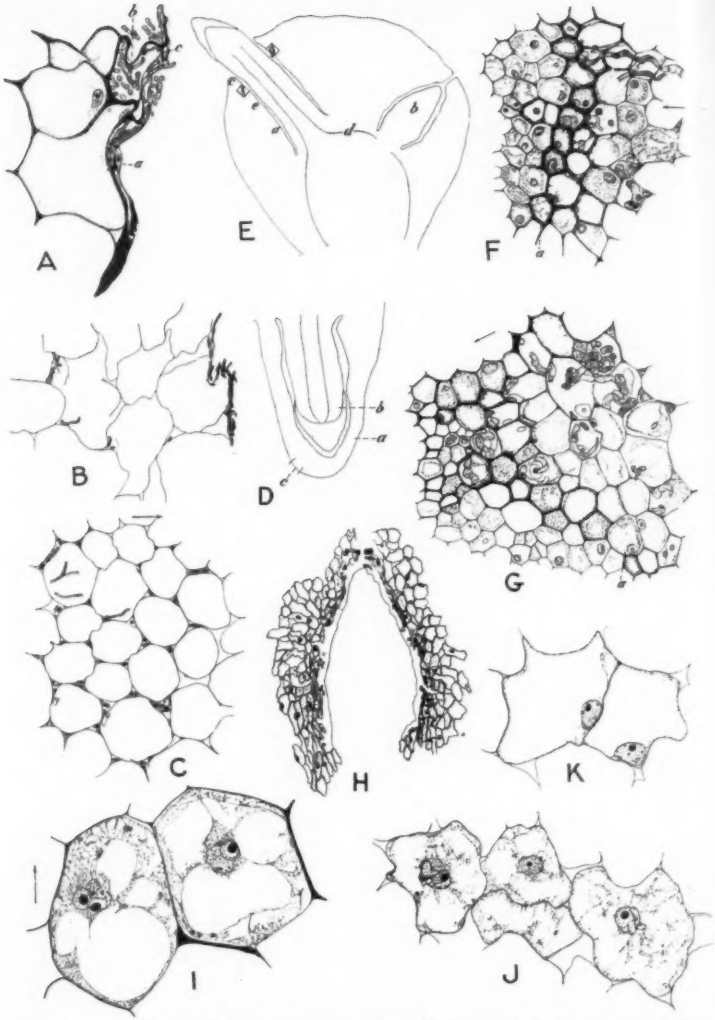

FIGURE 3.—A, Cells from the external margin of a rupture in the mesocotyl produced by root emergence. (E, *c*.) The fungus has established itself in the collapsed cells (*a*) and has entered an epidermal cell (*b*). × 252. B, Portion of the cortex adjacent to a rupture in the mesocotyl. (See E, *c*.) The fungus is growing in the rupture and has penetrated into the intercellular spaces of the cortex. No cells in this region have been invaded as yet. × 108. C, Portion of the cortex at the edge of a lesion showing intercellular invasion in advance of cell-wall penetration. × 108. D, Diagrammatic longitudinal section through an unruptured coleorhiza (*a*); *b*, young primary root; *c*, general location of the region from which other drawings were made. E, Diagrammatic cross section of the lower portion of the corn mesocotyl showing the relation of parts. The letters represent the regions from which other drawings were made. F, Portion of an infected mesocotyl at E, *d*. The fungus has progressed as far as the endodermis (*a*). Penetration is from cell to cell. The contents of cells in advance of penetration are granular. General plasmolysis of the tissue is due to fixation. × 108. G, Portion of an infected mesocotyl at E, *d*. (Very susceptible strain.) The fungus has penetrated the stele. *a*, Endodermis (?). A portion of the plesmolysis is due to fixation. The contents of cells in advance of penetration are granular. × 108. H, Cortical cells adjacent to a rupture in the mesocotyl produced by an adventitious root (E, *b*), showing conspicuous character of the cytoplasmic contents of cells bordering upon the rupture. × 58. I, Cells from an area adjacent to a lesion and just beyond the limits of fungus penetration. The stainable cytoplasm is distributed more or less throughout the cell. × 252. J, A few cells from near the base of a rupture (E, *a*). The stainable cytoplasm is distributed throughout the cell cavity. × 252. K, Two cells from "normal" tissue. The cytoplasm is in the form of a cytoplasmic sac. × 252 Arrows in C, F, G, and I indicate direction of fungus or of penetration

When only a few cells have been entered, the fungus has already penetrated into the intercellular spaces of the cortex to a depth of several cells. (Fig. 3, B.) As the lesion develops, intercellular invasion of the tissue is still seen to be several cells in advance of cell penetration (fig. 3, C); even in the center of a large section the hyphae tend to mass in the intercellular spaces rather than in the cell cavities (fig. 2, B). In regions where the cells are closely packed together and no intercellular spaces occur, as at the endodermis and in the stele, advance of the fungus is usually from cell to cell. (Fig. 3, F and G.) Hyphae are also capable of making their way between the cells (fig. 4, G), growth here evidently being accomplished by the dissolution of the middle lamella. A fourth and less frequent place where the fungus may be found to travel is in the walls of individual cells, as shown in Figure 4, I. Here the hyphae evidently are passing between two of the laminae of which the wall is composed.

The presence of the fungus in the host tissues appears to bring about a considerable increase in the thickness of the cell walls of certain rather definite regions. These regions will be more fully discussed later. However, it is in these thickened or swollen walls that the manner of fungus penetration from cell to cell is most easily observed. The hypha usually becomes greatly constricted at the point of penetration; a fine filament passes through the wall and immediately swells to its previous diameter upon emerging into the opposite cell cavity. (Fig. 5, C, D, and F.) This was observed only in the case of material preserved in Juel's fixative. When such material is stained with orange G and Delafield's haematoxylin, the walls of the host cells are yellow and those of the mycelium blue; the contrast in color makes the penetrating filament very conspicuous. Material fixed in medium chromo-acetic fixative has not proved at all satisfactory for a detailed study of the fungus in the host tissues. Instances in which the penetrating filament is easily distinguished occur in abundance in the walls of cortical cells near the endodermis and in the walls of what in some cases appeared to be the endodermal cells themselves. (Fig. 5, C.) Thickened or swollen walls occur in small lesions formed probably as the result of direct penetration of the epidermis, and here also the connecting filaments are very clear; two instances were found in which hyphae, present in the ruptures in the mesocotyl formed by root emergence, had penetrated into adjacent cortical cells of the mesocotyl. (Fig. 5, H.)

Not all the cell walls of the host tissues become exceedingly thickened or swollen as a result of fungus invasion; this is especially true of the walls of cells from the central portion of the cortex. In most instances these thinner cell walls appear to be penetrated in practically the same manner as the thicker ones; the hypha is usually deeply constricted on both sides of the wall, but a connecting filament can not be distinguished; it would be short and consequently difficult to see. (Fig. 5, I, J, and K.)

The question as to the exact means by which fungus hyphae penetrate cell membranes has been discussed since the time of De Bary. The greater part of the discussion has concerned the question of the penetration of cuticularized surfaces. Some study, however, has been made of the penetration of cell membranes within the host. De Bary (*6*), in a study of the parasitism of the broadbean by

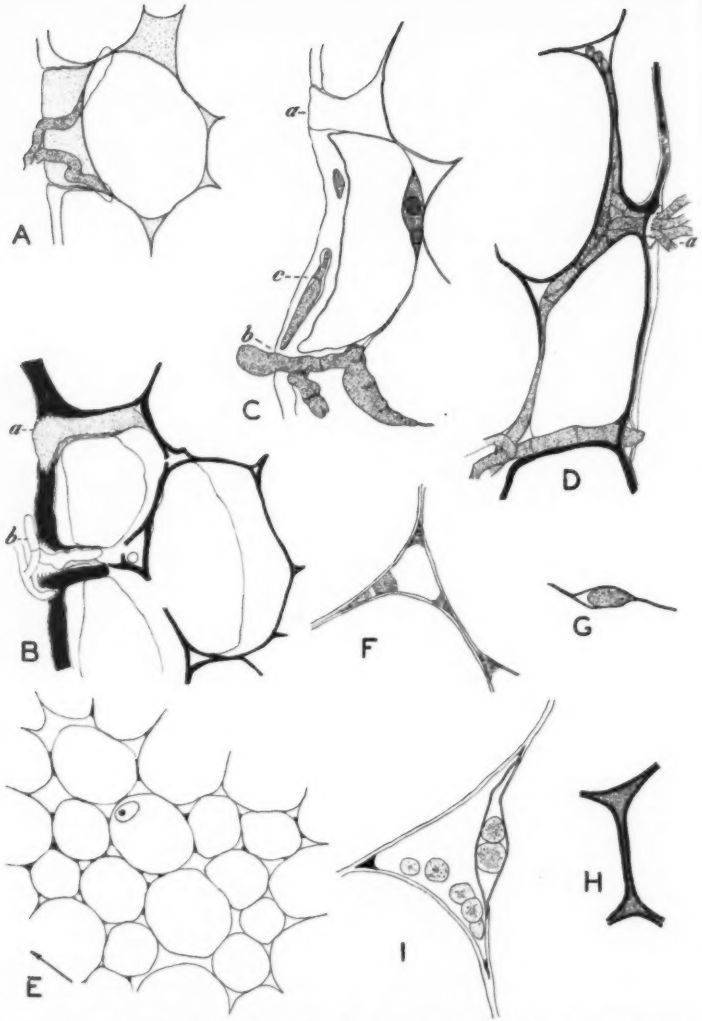

FIGURE 4.—A, Portion of the epidermis at the tip of the coleorhiza. (Fig. 3, D, *c*.) Two hyphae have entered one plug, whose material now consists of a yellow-staining mass. × 885. B, Portion of the epidermis of an unruptured coleorhiza. (Fig. 3, D, *c*.) A plug between two epidermal cells is shown at *a*. The fungus has entered a plug at *b*. The faint stippling surrounding the penetrating hypha represents what is probably the remains of the plug material. × 885. C, Portion of the epidermis at the tip of the coleorhiza. (Fig. 3, D, *c*.) A plug between two of the epidermal cells is shown at *a*. A hypha has entered at *b*. At *c* the fungus is seen to have established itself in the cell wall. × 885. D, Portion of the epidermis at the tip of the coleorhiza (fig. 3, D, *c*), showing penetration between the cells (at points formerly occupied by plugs). At *a* several hyphae have entered together and are surrounded by a "halo," which appears to consist of material that does not stain. A crack in this substance (due to cutting) shows the hypha beneath. × 885. E, Portion of the cortex at the edge of a large lesion and beyond the limits of fungus penetration, showing the accumulation of stainable material in the angles of the intercellular spaces. Arrow indicates direction of fungus or of penetration. × 174. F, A detail of E. An intercellular space showing the location of the stainable material. × 885. G, Portion of a cell wall. A hypha is making its way along the middle lamella. × 885. H, A thickened or swollen wall of a cortical cell near the endodermis. A portion of the thickness appears to be due to a swelling of the middle layers. × 885. I, An intercellular space showing cross section of hyphae which are traveling in the space itself and of hyphae which are growing between laminae of the cell wall. × 885.

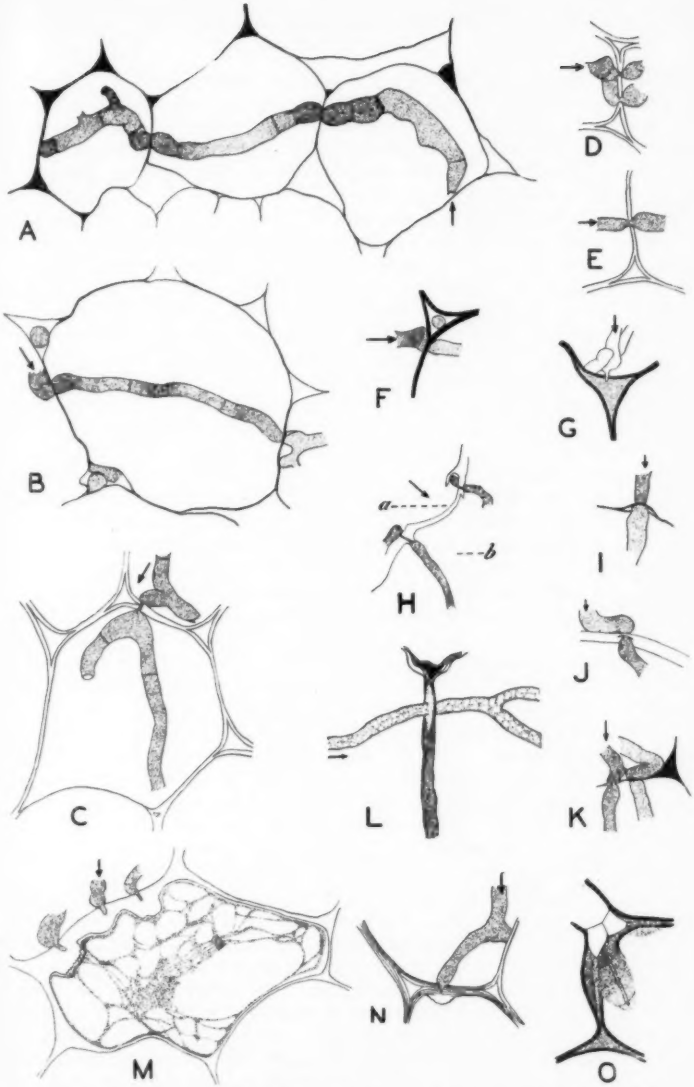

FIGURE 5.—A, Hypha growing through several cortical cells of the mesocotyl. × 885. B, Hypha growing through a cortical cell of an infected root. × 885. C, D, E, and F, Penetration of thickened cell walls. The hypha is greatly constricted, penetration taking place through a very small opening. × 885. G, An early stage in penetration. The hypha is located in a cell cavity and is penetrating into an intercellular space. × 885. H, Hyphae present in a rupture (*a*) have penetrated into adjacent cells of the cortex (*b*). × 885. I, J, and K, Examples of fungus penetration through comparatively thin cell walls in which the opening in the wall at the point of penetration is larger than the diameter of the penetrating hypha. × 885. L, Hypha growing through a cortical cell wall. A clear portion in the wall surrounds the hypha at the point of penetration. × 885. M, Callosities formed on the wall of a pericycle cell when penetration is attempted by hyphae present in an endodermal (?) cell. × 885. N, A callosity formed on the wall of a cortical cell near the endodermis. The wall is laminated, the callosity homogeneous. × 885. O, A large callosity formed on the wall of a hypodermal cell of an infected root. The callosity appears to have been formed by the addition of successive layers of material. × 885. Arrows indicate direction of penetration

Sclerotinia libertiana, concluded that the fungus secretes a substance or substances whose action on the underlying host tissues is twofold, (1) it softens the walls and (2) it kills the cells. He considered the substance acting upon the walls to be enzymic in nature. He was not sure of the character of the toxic substance but suggested that it might be a soluble oxalate.

Ward (40) studied the parasitism of *Botrytis cinerea* on lily and he also found an action on the host tissue in advance of fungus penetration. He concluded that the fungus secretes an enzyme which dissolves cellulose and which is capable of softening epidermal cell walls as well.

Other investigators, including Büsgen (*12*) and Voges (*39*), assumed that the cuticle is softened or dissolved by fungus secretions, the softening thus permitting penetration by the germ tube.

However, Miyoshi (*27*) demonstrated that hyphae are capable of penetrating membranes such as gold leaf, parchment, collodion films, and cellulose films impregnated with wax—materials upon which it seemed impossible for the fungus to act chemically.

One of the first to point out that previous investigators had not given careful consideration to the problem of cuticular penetration was Smith (*35*). He studied the parasitism of *Botrytis cinerea* on lettuce and as a result of his investigations concluded that the fungus produces some soluble substance, highly toxic in nature, which is capable of diffusing through the cuticle. The death of the underlying cells and the accompanying loss of turgor would render mechanical penetration of the cuticle possible. He considered the toxic substance to be oxalic acid, since this substance was found in the mycelial extract and since in dilute solutions pure oxalic acid produced results similar to those caused by the extract.

Brown (*9*) injected a carefully prepared mycelial extract of *Botrytis cinerea* into disks of fleshy tissue and found that it produced (1) a macerating action on the walls and (2) death of the cells. From a series of experiments he concluded that only one substance, probably enzymic in nature, is responsible for these actions.

Blackman and Welsford (*7*) studied the method of infection of *Vicia faba* by *Botrytis cinerea*. They concluded that passage of the fungus through the cuticle is entirely a mechanical process. This conclusion is drawn from the facts (1) that the ends of the germ tubes seem to be exerting a pressure, as indicated by their flattened appearance and by the slight indentation of the host cell wall beneath them; (2) that the cuticle appears in no way altered; and (3) that no change in the subcuticular layers occurs until after the cuticle is ruptured. They do not discuss the method of penetration of pure cellulose walls, but admit the production by the fungus of a substance which is capable of acting upon cellulose, as is suggested by the alteration in the subcuticular layers of the epidermal walls following penetration of the cuticle.

Similar observations have been reported by Dey (*13*) in the case of the infection of bean pods by *Colletotrichum lindemuthianum*, by Boyle (*8*) in the infection of bean leaves by *Sclerotinia libertiana*, and by Waterhouse (*41*) in the infection of leaves of *Berberis vulgaris* by the sporidia of *Puccinia graminis*.

Brown (*10*) also concluded, from experiments with mycelial extract and infection drops placed on cuticularized surfaces, that the fungus

secretes neither a substance capable of acting upon the cuticle nor any toxic substance capable of diffusing through it; therefore cuticular penetration must be by mechanical means.

Hawkins and Harvey (*20*) made careful observations of the actual growth of the hyphae of *Pythium debaryanum* through the cortical cell walls of potato tubers. The manner in which the hyphae were bent and the enlargement at the point of contact with the host cell wall led them to conclude that penetration is mechanical.

Leach (*25*) reports that at early stages in the infection of stems of susceptible bean plants by *Colletotrichum lindemuthianum* the hyphae appear forcibly to pierce the cell walls . Later it seems evident that there is an enzymic action which softens the walls.

Brown and Harvey (*11*) inoculated the surfaces of leaves of Eucharis with spores of *Botrytis cinerea*. No penetration was observed to occur unless the turgor of the underlying cells was reduced. Penetration, they hold, can not be explained as the result of enzymic action, since a reduction in turgor of the host cells would have no effect on such a process. They conclude that the "only possible basis of explanation is the mechanical theory of penetration." They explain penetration by the effect of a contact stimulus upon the fungus.

In the study of lesions on the corn mesocotyl no instances were observed of an actual piercing of the epidermal wall by the fungus. Small lesions, in which only a few cells are invaded, appear to result from direct epidermal penetration, but the early stages of such a process were not found.

A study of the numerous examples of penetration through cortical cell walls does not furnish evidence sufficient to justify the conclusion that penetration is either by a chemical or by a mechanical process alone. Hyphae were observed closely appressed to the wall and enlarged previous to constriction (fig. 5, F), as though they were exerting a pressure; yet many instances were observed in which there was little or no enlargement at this point (fig. 5, E). Hyphae also appeared bent. (Fig. 5, A and B.) Bending is believed by Leach (*25*) and Hawkins and Harvey (*20*) to occur when a hypha extends across a cell cavity and, in attempting to penetrate the opposite wall, meets with resistance. Hyphae growing through several cell cavities (fig. 5, A) or entirely through one (fig. 5, B) were carefully studied; and the manner in which each hypha extends across the host cell, although it is somewhat bent, does not suggest the rigidity necessary for the fungus to rupture the cell walls of the host by mechanical means alone. Examples of penetration such as the one shown in Figure 5, G, certainly would not support a theory of mechanical action alone, for, if pressure were being exerted, the enlargement of the hypha would of necessity be pressed against the host cell wall. Indentations of cell walls at points of penetration might conceivably occur in cases of mechanical rupturing of thin cell walls, but no convincing examples of this nature were observed.

There is some slight indication that a chemical action occurs at the point of penetration. In material preserved in Juel's fixative, a few of the host cell walls were cut at such an angle that a more or less perfect surface view of the wall was obtained at the point where penetration occurred. In several cases the opening in the wall at this point was larger than the diameter of the penetrating portion of the fungus

hypha. (Fig. 5, I, J, and K.) Such an opening suggests a dissolution of the material at this point. However, it is possible that, in the piercing of a fairly taut membrane such as the cell wall, a slit might be made at the point of rupture. Further evidence in favor of a chemical action was obtained from a series which had been preserved in medium chromo-acetic fixative. The most striking example of this nature is shown in Figure 5, L. Here, also, practically a surface view is obtained at the point where the fungus penetrates the cortical cell wall. In this instance that portion of the wall surrounding the point of penetration is relatively clear. The clear area seems to represent, not a dissolved portion of the wall, but rather a region that takes up no stain. The hypha seems not to be constricted, but this appearance may result from the angle at which it is viewed. The "halo" certainly could not be explained on the basis of a purely mechanical process. The fact that medium chromo-acetic fixative usually gives a good fixation makes it seem reasonable to suppose that the halo represents a chemical change in the wall substances accompanying penetration.

A large portion of the cell walls of the susceptible corn plants, and of those of the more resistant ones grown at the lower temperatures, is composed of pectinlike materials, substances on which the fungus can act chemically and which can be used as food (*14*, *15*, *16*). An enzyme secreted by the fungus may so soften the walls that they can be pierced by the hyphae with very little effort. The greatly thickened or swollen walls met with so frequently probably do not offer so much resistance as might be supposed at first glance; the chemical action on the walls in these instances may result in the production of substances that swell considerably and that, although they appear thick, offer no more resistance or even less than thinner walls.

There appears to be some evidence that penetration is at least in part a chemical process; this evidence, however, is slight, the above-described conditions not being of widespread occurrence. Very probably it is not a question of one method or the other; both methods may be concerned in bringing about the passage of the fungus through the cell walls of the host. And, further, the wall composition of the host plants may play an important rôle in determining which method will predominate. For example, the pectinlike and xylanlike cell walls of the low-temperature seedlings, easily hydrolyzed by the parasite, undoubtedly are acted upon chemically in wall penetration, whereas true cellulose walls are less subject to chemical action by this parasite and their penetration may involve more or less mechanical action.

Penetration of the Coleorhiza

An examination of the tip of the coleorhiza reveals the presence of pluglike structures between the epidermal cells. (Fig. 1, C.) These probably result from the deposition of material into spaces formed by the pulling or tearing apart of the epidermal cells of that region during the rapid elongation and expansion of the apical portion of the coleorhiza during seedling growth. The plugs are distinct from the walls, each being bounded, in section, usually by a light line, which may indicate a slight shrinkage of the plug material during fixation. The outlines of the plug and the edges of the walls are frequently very

jagged. (Fig. 4, B.) This appearance suggests a tearing apart of the wall at this point and a later filling in of the cavity.

The epidermal walls at the tip of the coleorhiza are thick and appear to be composed, apart from the cuticle, of two layers, an outer layer which stains blue with Delafield's haematoxylin and an inner layer which may be the true cellulose wall. (Fig. 4, B.) In uninoculated material the plugs appear similar in their staining capacity to the outer layer. It has not been determined with certainty whether or not these structures are cuticularized, but the evidence available seems to indicate that at the low temperatures or in susceptible seedlings they are not cuticularized.

When material that has been inoculated upon the surface of the coleorhiza is examined (even after 24 hours), numerous cases of penetration are observed. Every entrance appears to have been effected where a plug developed, and in some sections nearly every plug in the inoculated region has been entered. (Fig. 1, D.) Penetration at these points is obviously a very rapid and easy matter. The entrance of the hypha into the plug is not accompanied by the formation of a small penetrating filament such as is described by Blackman and Welsford (7) and others in cases of cuticular penetration. Little or no constriction of the hypha is noticed at the point where penetration occurs. (Fig. 4, C and D.) The fungus grows into these structures as though passing into a substance that offers little or no resistance to penetration.

Changes in the staining reactions of the plugs indicate that they are probably acted upon chemically by substances secreted by the fungus. Plugs in the inoculated region tend to stain yellow rather than blue. Their substance apparently becomes more or less liquefied, for in their places frequently appear large yellow-staining amorphous masses. (Fig. 4, A.) Other instances have been observed in which the penetrating hypha is surrounded with a faintly stained material in the region formerly occupied by the plug. (Fig. 4, B, *b*.) In one clear case the penetrating hyphae were surrounded by a halo. (Fig. 4, D.) In this instance several hyphae had entered at the point where a single plug developed. This clear area appeared not to be the result of the dissolution of material or of plasmolysis, but indicated rather the presence of a substance that did not stain. It is obviously cracked in one place (probably by the action of the microtome knife), and the hypha below may be seen. Possibly at some stage in the dissolution of the plug material a substance is formed which does not stain. This halo is similar to the colorless area described in the case of a penetration through a cortical cell wall of the mesocotyl. (Fig. 5, L.) It may be concluded that the plug is composed of some substances that are readily dissolved by the fungus, and its apparent decrease in quantity may indicate that it serves as food for the fungus.

The fungus not only progresses inward through the intercellular spaces of the coleorhiza but also establishes itself in the outer layer of the epidermal wall. (Fig. 4, C, *c*.) The presence of the fungus in this layer causes a considerable swelling and a tendency, after invasion, to stain a light orange brown with orange G.

Microchemical tests have shown that the outer layer of the epidermal walls of the mesocotyl and the upper portion of the coleorhiza of susceptible plants grown at low temperatures is composed of a xylan-

like substance.[7] It seems logical to conclude that the same substance comprises the blue-staining outer layer of the epidermal cell walls at the tip of the coleorhiza. The resemblance, in their staining capacity, of the plugs to this outer layer would suggest that they consist of a similar substance. The probable xylanlike nature of this outer layer of the wall, of the middle lamella, and of the plugs would indicate that the changes occurring in these structures are alterations in their composition due to the action of the fungus secretions, since it has been established that the fungus may use xylanlike substances as a source of carbon (*16*). Little cellular penetration occurs until the coleorhiza tip is thoroughly invaded, sufficient food evidently being found in the materials of which the host cell walls are composed, or possibly in substances exuded from the cells themselves after the changes in the physical state of the cytoplasm brought about by fungus secretions. The fungus may progress from the invaded coleorhiza into the primary root and destroy the root before it emerges.

Penetration of the epidermis of the coleorhiza appears to be entirely a chemical process, but it must be borne in mind that the fungus gains entrance through natural openings which are probably not cuticularized.

FUNGUS ACTION ON THE HOST CELLS

Reactions Apparently Due to Direct Action of the Fungus or Its Secretions

Fungus invasion produces various modifications in the character of the cells of the host tissues. These modifications may be alterations in the chemical nature of the walls and of the protoplasm, due to the direct action of the fungus secretions; they may be responses on the part of the host plant adapted to check or prevent further penetration by the parasite; or they may be in the nature of wound responses.

The most conspicuous change in the host tissues is a markedly increased tendency for the cell walls to take up considerable stain. This change is most conspicuous in material fixed in the medium chromo-acetic fixative. When a large lesion is examined under the low power of the microscope the region of densely stained walls is seen to extend for a considerable distance beyond the limits of fungal penetration. (Fig. 2, D; fig. 6.) In sections that have been partly destained a series of changes may be observed in the staining capacity of the walls of the cells of this region. The first visible change in the walls of cells at the greatest distance from the fungus is the accumulation of stain in the angles of the intercellular spaces. (Fig. 4, E and F.) This is followed by the accumulation in the intercellular spaces themselves of globules which stain darkly (fig. 2, A) and which evidently increase in volume, for, with a closer approach to the area occupied by the fungus, entire intercellular spaces may become filled with the dark-staining globular mass (fig. 2, A; fig. 6, C). This area merges into that in which the fungus has established itself, and here the entire walls are heavily stained and practically every intercellular space is filled with a mass of dense amorphous material. (Fig. 2, D.) The walls in an extensively invaded area show a diminished affinity

[7] These tests were performed by J. G. Dickson and consist in brief of the following method of procedure: The presence of these substances was demonstrated by solubility tests. The pectinlike materials were dissolved out by a hot 0.5 per cent ammonium oxalate solution. Following this the xylan materials were dissolved out by a hot 1 per cent sulphuric acid solution

for stains, and in the center of a large lesion they appear relatively thin, are not deeply stained, and there is less material in the intercellular spaces. (Fig. 2, B.)

Accompanying these changes in the staining reactions of the cell walls are changes in the protoplasm itself. These changes may be considered to be the first effects of the fungus secretions on the host tissues, for they occur beyond the area in which the walls are stained deeply and therefore at a greater distance from the fungus. These reactions, which will be discussed at greater length later, consist in (1) an orientation of the nuclei toward that side of each cell nearest the lesion (fig. 6, A and C) and (2) changes in the character and distribution of the stainable portion of the cytoplasm. This stainable portion is more conspicuous than in other cells; it stains more deeply, appears to have increased in amount, and, though decidedly vacuolated, is more or less distributed throughout the cell cavity with its

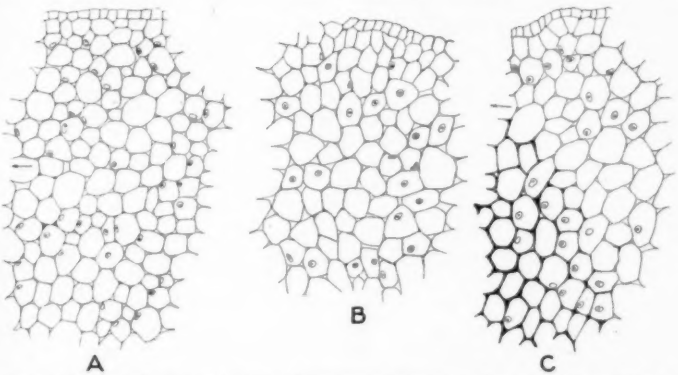

FIGURE 6.—A and C, Regions adjacent to large mesocotyl lesions. The nuclei of the majority of the cells are seen to be located in that portion of the cell which is nearest the lesion. B, Portion of normal tissue showing nuclear distribution. × 51

greatest bulk in that half of the cell which is nearest the lesion. (Fig. 3, I.)

The first effect of the presence of the fungus or of its secretions upon the host tissues may be in the nature of a wound stimulus, the cytoplasm responding in such a manner as to produce the visible changes already described. It is conceivable that these responses may precede or accompany some physicochemical changes on the part of the protoplasm, which result in the secretion of the materials into the intercellular spaces or their accumulation in the cell walls.

Chemical tests have shown that susceptible corn seedlings and all corn seedlings grown at low temperatures contain substances that yield pentoses on hydrolysis. These substances form part of the cell walls and may include reserve materials in the cytoplasm as well (*14, 15, 16*). It has been demonstrated that the fungus grows readily on pectic substances and pentosans from corn seedlings, proving that it does produce enzymes capable of hydrolyzing the compounds found in abundance in the susceptible and low-temperature corn seedlings.

The changes in the staining reactions of the walls, and the accumulation of materials with a similar staining capacity in the intercellular spaces, could then be interpreted as representing a hydrolysis of pectinlike materials and pentosans present in the cell walls, or of similar substances deposited in the walls and intercellular spaces by the cytoplasm, the products of this hydrolysis being compounds that stain very readily. The fact that there appears to be less of this stainable material in the center of a large lesion where the fungus is very abundant would suggest that a portion of it may have been used later by the fungus as food.

On the other hand, the secretion of substances from the protoplasm possibly may be considered as a means of blocking further progress of the fungus, for at the higher temperatures and in resistant strains this secretion seems to be followed by the suberization of the host cell walls in this same area (*14, 16*). But in the susceptible strains at the low temperatures, although the accumulated products appear dense, they do not seem in any way to check the advance of the parasite, for the hyphae may be seen growing in the densely stained masses in the intercellular spaces.

Johann (*21*) describes a similar accumulation of materials in the walls and intercellular spaces of cells surrounding lesions produced by *Penicillium oxalicum* in corn mesocotyls.

Allen (*4*) reports the formation of similar globules or "warts" in the case of the infection of Malakoff wheat by *Puccinia triticina* physiologic form 11. She finds that these structures take up stains that are generally supposed to stain pectic materials, and concludes that they may be the result of a hydrolysis of the middle lamellae of the host cells, the enzyme responsible probably being produced by the fungus.

Smith and Walker (*36*) report browning and plugging of vascular elements in cabbage plants susceptible to and attacked by *Fusarium conglutinans*. In comparable resistant cabbage plants the plugging occurred only occasionally.

Many investigators assume that an enzyme capable of dissolving the middle lamellae is produced by various fungi and bacteria. That such a substance is produced has been demonstrated in the case of *Bacillus carotovorus* (*22*), *Rhizopus tritici* (*19*), and *Botrytis cinerea* (*9*). In the majority of instances the apparent value of the enzyme to the fungus lies in the fact that it aids in penetration rather than that it directly provides food substances. But in the case of the infection of the corn plant by *Gibberella saubinetii* the possibility of a nutrient relationship should not be disregarded.

A toxic influence is observed ahead of cell penetration in the cortex, the host cells becoming plasmolyzed about two or three layers in advance of those occupied by the fungus. At the endodermis, where advance is mainly from cell to cell rather than through intercellular spaces, this toxic action is more pronounced. For two or three layers of cells in advance of cell penetration the cell contents are very granular and appear to be precipitated. (Fig. 3, F and G.) The cells of this region contain a greater amount of cytoplasm in proportion to their size than do those in the central portion of the cortex and remain physiologically active longer—facts which may account for the more conspicuous changes in their contents.

Material fixed in Juel's fixatives presents a slightly different appearance. The globules and masses of stainable material (staining yellow with orange G) are present in the intercellular spaces and are similar in appearance to those found when material is fixed in the medium chromo-acetic solution. In addition, however, thickened or swollen walls are found to be associated with invasion. In some instances it frequently appears to be the entire wall that is swollen, and laminations may be very pronounced. (Fig. 5, N.) In other cases careful examination indicates that the greater portion of the thickness is due to the increase in the size of the middle layer. (Fig. 4, H.)

There may be several explanations for the increase in thickness of these walls. It may be that the middle lamella, which in certain tissues is of a pectinlike material, under the influence of certain enzymes possesses the ability to swell to a considerable extent during hydrolysis. The fungus is able to act upon the materials of which the middle lamella is composed (*14*, *16*), and the products of its action may be substances capable of considerable swelling, this characteristic being more sharply brought out in Juel's than in the chromo-acetic solution. And again, the cell walls of susceptible plants and of all those grown at low temperatures may also possess considerable pectinlike and xylanlike substances in which a similar swelling might occur, causing definite thickening of the walls, the combined action resulting in such greatly thickened walls as are shown in Figure 1, B. It is possible also that the increase in thickness may be due in part to an accumulation in the cell walls of products secreted from the cytoplasm and to the hydrolysis of such compounds. These thickened or swollen walls are not of universal occurrence throughout the lesion, but seem to be more or less limited to areas where secondary thickenings or deposits are likely to be made, such as the hypodermal layers of the mesocotyl, cells in the vicinity of the endodermis, including cortical as well as pericycle cells, and cells lining the root ruptures. In all these regions secondary deposits in the walls, such as suberin or lignin, are deposited under favorable environmental conditions. At low temperatures the complete formation of these substances does not take place (*14*, *15*, *16*), and if secondary depositions do occur they would probably consist of pectinlike materials or pentosan compounds upon which the fungus can act chemically and which may also possess the ability to swell. As a rule, no greatly thickened walls occur in the central portion of the cortex of the mesocotyl; however, they are found in the cortical cells of the coleorhiza. (Fig. 1, E.) The thickened walls, therefore, may be accounted for in two ways, namely, by the hydrolysis and swelling of existing wall substances, or by the hydrolysis of substances laid down as preliminary barriers in partial response to the fungus invasion.

Reactions Which May be Manifestations of Resistance

Dickson and Holbert (*16*) have shown that the endodermis, as well as one or two layers of the pericycle, in resistant lines of corn seedlings, even at fairly low temperatures, is well suberized, and may thus act as a barrier against invasion from the cortex into the stele. Seedlings grown at very low temperatures and susceptible seedlings have only a patchy or incomplete suberization of the endodermis. A

section of the endodermis in a semiresistant strain is shown in Figure 1, B. Here the advance of the fungus is seen to be checked, temporarily at least, and it is massed in the cell cavities. The suberin deposited in the walls may be effective in two possible ways: (1) Its presence in the middle lamella may prevent penetration of this layer by the fungus, either in its growth between the cells or in a passage from one cell to the next; (2) its presence may deprive the fungus of sufficient suitable food and thus check its progress. That resistance is not complete is shown by penetrations into the endodermal cells and even into those of the pericycle. (Fig. 3, G.)

In extremely susceptible material the stele is entered more frequently, and yet even here the endodermal region appears to offer some resistance. (Fig. 3, F.) The exact determination of the endodermis by staining reaction in such material can not always be made with certainty. In the region of the mesocotyl, just above the cotyledonary plate, an endodermis can not always be distinguished, or it may not have become differentiated; hence it is quite possible that the cells that offer resistance in such regions are actually those of the pericycle.

The production of callosities indicates that the cells may exhibit a still more specific type of resistance to penetration. Such a structure is shown in Figure 5, N. A penetrating filament enters the wall, while on the opposite side of the wall a swelling or wall thickening is produced. This swelling is usually rather rounded in general outline, but frequently its margin is jagged. (Fig. 5, M.) Such structures occur abundantly in the cortical cells near the endodermis, in cells of the endodermis and pericycle, and in hypodermal cells of the cortex, especially of the root. Thus the swellings appear to be associated with the cells that tend, under normal environmental conditions, to have secondary depositions within their walls. No callosities were found in the central portion of the cortex, which is much less active in every respect than the hypodermis, the endodermis, or the pericycle. The substance of the callosities formed in the susceptible or intermediate strains appears to be, in general, the same as that of the wall. They stain with the same stain but may be less dense. (Fig. 5, N.) Frequently the wall is laminated, but in such instances the callosity usually appears homogeneous.

Smith (*34*), in discussing the formation of haustoria in certain of the Erysipheae, describes a pluglike structure which is formed on the host cell wall and incloses the invading hypha for a short distance; this he considers to be formed by the addition of new wall material. Young (*42*) reports the extensive formation of callosities when wheat coleoptiles and stems of other seedlings are inoculated with Alternaria, Diplodia, and other fungi. Similar structures were observed by Fellows (*17*) in the infection of wheat by *Ophiobolus graminis* and by Simmonds (*33*) in the infection of oats by *Fusarium culmorum*. The callosities formed vary considerably in size from small rounded ones, which are occasionally toothed, to elongated structures which follow the penetrating hyphae for considerable distances. Young (*42*) and Simmonds (*33*) do not definitely state what they consider to be the method of formation or the chemical composition of these structures. Fellows (*17*) found them to respond to tests for lignin.

Not enough evidence has been accumulated in the case of Gibberella infection on corn to permit one to make a definite statement as to the

probable method of formation of callosities. A difference in density between wall and callosity could be interpreted as the result either of a swelling of material already present or the addition of new material; but examples occur in which the wall and callosity appear as one, and in such cases it seems impossible to assume the addition of new material. Differences in density could very possibly be attributed either to differences in the amount of infiltration or to the amount of chemical change in the wall material at that point. In the example shown in Figure 5, O, it appears as though the structure were the result of the continued deposition of several layers of new material.

With but one possible exception, no hypha was observed to grow out of a callosity. This exception appeared in the case of a penetration from a root rupture into a cell of the cortex. (Fig. 5, H.) If this slight swelling is a callosity, it is a very small one. Young (*42*) and Fellows (*17*) report the final emergence of penetrating hyphae in some instances, but a failure to emerge in others.

Wound Responses in the Plant

A study of the host tissues adjacent to a fungal lesion shows the majority of the nuclei to be oriented toward that side of each cell nearest the lesion. A similar response was observed in the cells bordering on the ruptures in the mesocotyl produced by the emergence of the adventitious roots.

A traumatotaxis of nuclei was first observed by Tangl (*37*). He worked with *Allium cepa* and noticed a movement of the nuclei and cytoplasm toward the side of each cell nearest the wound. Nestler (*28*) demonstrated the general occurrence of this phenomenon, finding it in all plants and organs studied. These observations were confirmed by Miehe (*26*), working with the epidermis of leaves of Allium and stems of Tradescantia and Tinantia; by Schürhoff (*31*), working with the epidermis of leaves of Tradescantia; and by Ritter's (*30*) investigations on the epidermis of bulb scales of *A. cepa*.

As the reactions in the corn plant resembled the wound reactions described by earlier workers, a study of these responses was made. Reactions to three types of wounds were considered: (1) Lesions in the mesocotyl produced by fungus invasion, (2) ruptures in the cortex of the mesocotyl caused by the emergence of the adventitious roots, and (3) needle pricks in the mesocotyl.

FUNGAL LESIONS

If the cells from the region adjacent to a lesion are compared with those at some distance away, two differences in the protoplasmic content and its distribution are noted. In normal tissues [8] the stainable cytoplasm is in the form of a peripheral layer with the nucleus located on no definite side of the cell. (Fig. 3, K.) However, an examination of the cells at the border of the lesion shows that the majority of the nuclei have moved toward the side of each cell nearest the lesion. (Fig. 6.) The stainable portion of the cytoplasm is also different in character from that of the normal cells. It stains more deeply and appears to have increased in quantity.

[8] The term "normal" is used to characterize the cells of tissues that are at a considerable distance from the wound or fungus and which therefore are probably uninfluenced by the stimuli produced as a result of mechanical wounding or of fungus invasion.

Although decidedly vacuolated, it is distributed more or less throughout the cell cavity, with its greatest bulk in the half of the cell that is nearest the lesion. (Fig. 3, I.) These reactions occur in a region several cells beyond the area occupied by the fungus and even beyond the area in which the cell walls show a change in their staining reaction. (Fig. 2, D.)

The nuclear orientation is more strikingly brought out in a chart (fig. 7), which illustrates the nuclear distribution in normal tissue (B) and in a region bordering a lesion (A). This chart was made as follows: The nuclei from selected fields of microscopic vision were plotted according to their positions in the cells. If a nucleus was close to the wall toward the lesion, a dot was placed in column 1; if located near the opposite wall, a dot was placed in column 7; if located in the center of the cell, the dot was placed in column 4; the numbers 2, 3, 5, and 6 indicate intermediate positions. Each dot on the chart thus represents a nucleus, and each single row of dots across the chart represents the number of nuclei visible in the two fields of microscopic vision from one section of the series on a microscopic slide. Consecutive rows of dots represent successive sections of the same mesocotyl. Two neighboring fields from each section were used, for the number of nuclei visible in one field of vision was too small to render the plotting significant. The two fields selected were side by side, and in the case of a lesion the two fields were at the edge of the area exhibiting a change in the staining character of the cell walls. (Fig. 6, C.) By comparing B in Figure 7, which represents the nuclear distribution in two such fields at a considerable distance from the region penetrated by the fungus, with A, which represents the distribution in two similar fields adjacent to a lesion, it is seen that the majority of the nuclei that normally occupy the positions in the cell represented by Nos. 5, 6, and 7 (away from the lesion) have moved to the positions represented by Nos. 1, 2, and 3; that is, toward the side of the cell nearest the lesion.

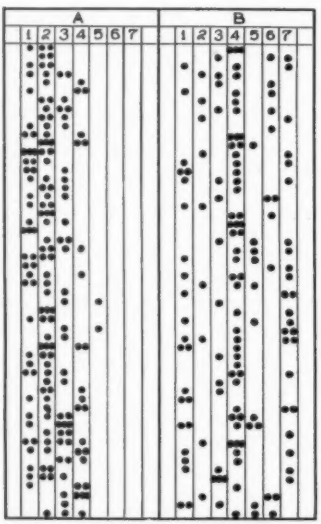

FIGURE 7.—A, Nuclear distribution in tissue adjacent to a fungal lesion; B, nuclear distribution in normal tissue

The influence of the fungus on the host protoplasm, as exemplified by the nuclear response, extends to some distance in a transverse direction (fig. 6), but its limits could not be determined. That a considerable influence is exerted in a longitudinal direction is shown by Figure 8. This chart was prepared like the previous one. The observations upon which it is based were made upon a series of transverse sections through the upper portion of an elongated lesion on the mesocotyl, including also some of the normal tissues above it. Two fields were selected from each section; when normal tissues were

reached, care was taken to select fields as nearly as possible from the same general region of the cross section of the mesocotyl as that from which the fields in the lower portion of the mesocotyl were chosen. The lowest line of dots on the chart represents the lowest section through the mesocotyl; the uppermost line, the highest section through the mesocotyl. Nuclear orientation toward the lesion is clearly demonstrated in the basal portion of the chart. The region of nuclear response is seen to continue for a considerable distance beyond the limits of fungus penetration and finally merges into one in which the nuclear distribution is undoubtedly normal.

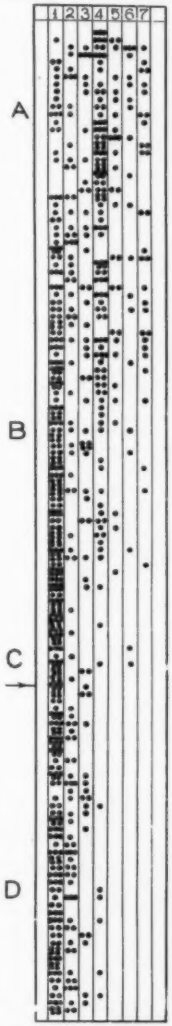

FIGURE 8.—Extent of fungal influence as indicated by nuclear distribution: A, nuclear distribution in tissues beyond the limits of fungal influence (the distribution is apparently that of normal tissue); B, tissue beyond the limits of fungus penetration but still showing the influence of the fungus on nuclear distribution; C (arrow), ending of the fungus; D, nuclear distribution in tissue adjacent to the lesion

RUPTURES OF THE CORTEX PRODUCED BY ROOT EMERGENCE

Responses resembling those described for the cells adjacent to the lesion also occur in the cells bordering on the ruptures in the cortex produced by the emergence of adventitious roots. Cells with conspicuous contents are frequently found to be associated with these wounds. (Fig. 3, H.) These cells are usually located near the base of the rupture as seen in cross section (fig. 1, A) and are apparently linked with the early stages of secondary root growth and rupture through the cortex, for they are not found to occur when the roots are older and the walls of the cells lining the ruptures have been appreciably suberized. (Fig. 2, C.) The stainable portion of the cytoplasm is scattered throughout the cell cavity (fig. 3, J) instead of being in the form of a peripheral layer characteristic of the mature cells of the cortex (fig. 3, K), and appears to have increased in amount. The nucleus is located near the center of each cell and usually does not exhibit a striking wound response; it may, however, have responded earlier and returned to the center of the cell. Ritter (*30*) studied nuclear behavior in living tissue and found that when wounds were made in a transverse direction the nuclei, after reaching the position of maximum reaction, usually returned after a certain length of time to their normal position.

An examination of the outer portion of a rupture surrounding a young root (fig. 1, A) or of the entire surface of an older rupture (fig. 2, C) shows that here there is a decided tendency for the majority of the nuclei to arrange themselves on the side of each cell toward the rupture. Charts made by plotting the nuclear distribution in fields of microscopic vision adjacent to the rupture, when compared with charts representing normal nuclear distribution, demonstrate the extent of this response. (Fig. 9.) Each line across the chart repre-

sents two fields of microscopic vision selected at opposite sides of the root. (Fig. 3, E, *f*, *g*.) Each line of dots in the chart showing normal nuclear distribution also represents two fields, one on either side of, but at some distance from, the rupture. As the walls of the cells lining the rupture finally become more or less suberized (*16*), suberization apparently beginning in the outer portion, it might be assumed that this nuclear position is associated with suberization. Haberlandt (*18*) finds that in many cases a nucleus tends to occupy that portion of the cell in which there is the greatest metabolic activity; for example, a nucleus lies near a wall that is being covered with cutin. Suberization would likewise increase the metabolic activities in the cytoplasm beneath the wall concerned, and the nucleus might tend to assume a position on that side of the cell. This may in part account for the decided nuclear movement, especially in the cells bordering directly on the rupture; but it is not the entire explanation, as is shown by comparing A and B in Figure 9. Both A and B are plotted from the same areas, but in the case of B the nuclei in the layer of suberized cells bordering on the rupture are omitted. A nuclear response is still very evident. It is scarcely probable that such far-reaching effects are the indirect result of a suberization of the outermost cell walls. They are more likely due to some other stimulus, possibly a "wound stimulus." However, it is difficult to understand why the nuclei of the cells with conspicuous contents (fig. 3, H and J) which are located at the base of the younger ruptures in the mesocotyl (fig. 1, A) fail to exhibit this response.

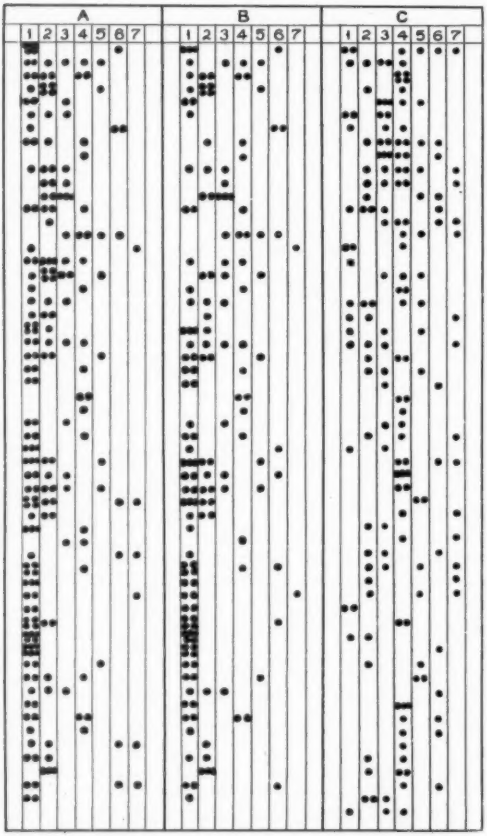

FIGURE 9.—A, Nuclear distribution in tissues adjacent to a rupture in the mesocotyl produced by root emergence; B, nuclear distribution of the same area; the nuclei of the suberized cells bordering on the rupture are omitted; C, nuclear distribution in tissues at some distance from the rupture

NEEDLE-PRICK WOUNDS IN THE CORTEX

Wounds were made in the mesocotyl of the young corn seedlings by means of pricks with a sterile needle; the portions including the wounds were fixed after 6, 24, 48, and 72 hours and after 5 days. Stained sections of the 6, 24, and 48 hour and 5-day material were examined, and no changes in the character of the cytoplasm similar to those occurring in the cells bordering on the root ruptures and fungus lesions were observed.

The production of a nuclear response, however, is apparent when a comparison is made between the nuclear distribution in the normal tissues and the wounded tissues. (Fig. 10.) Microchemical tests (*16*) have shown that the cell walls surrounding these wounds also become suberized; here, therefore, as in the case of the root ruptures, in order to determine whether a nuclear response occurred independently of suberization, a chart was made omitting the nuclei from the layer of suberized cells lying next the surface of the wound. (Fig. 10, B.) A nuclear response is seen to occur here as in other similar instances.

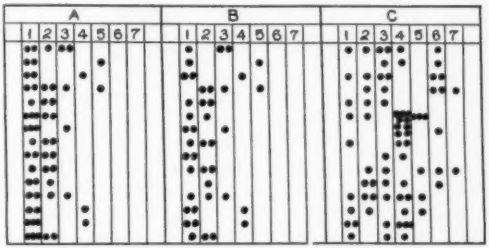

FIGURE 10.—A, Nuclear distribution in tissues adjacent to a needle-prick wound in the cortex of the mesocotyl; B, nuclear distribution of the same area; only the nuclei of the suberized cells bordering on the wound are omitted; C, nuclear distribution in tissues at some distance from the wound

DISCUSSION OF WOUND RESPONSES

Thus it is seen that nuclear reactions similar to those reported by earlier workers in response to mechanical injuries were induced by these three types of wounds. Alterations in the cytoplasm, either in appearance or in distribution, occurred, however, only in the cases of fungal lesions and of cortical ruptures due to root emergence.

Nuclear responses to the stimulus of a parasite have been reported in numerous cases. Riker (*29, p. 31*), in a study of the crown-gall tissue, finds that "the orientation of the spindle in the dividing cell appears to be influenced in the early divisions after inoculation by the position of the bacteria."

Allen (*1, 2, 3*), in her cytological study of infections of several varieties of wheat by certain strains of rusts, observed in many cases that the nuclei and a large portion of the stainable cytoplasm were displaced toward the haustoria.

Blackman and Welsford (*7*) describe a movement of the nuclei of the palisade cells of bean leaves to the upper portions of the cells after an invasion of the epidermis by *Botrytis cinerea*. Boyle (*8*) observed a similar occurrence in bean pods infected with *Colletotrichum lindemuthianum*.

Both mechanical wounds and fungus invasion may induce the formation of new tissues. Wounds may cause the formation of callus and wound cork. Cork layers may be formed. A case of the latter

sort is described by Tisdale (*38*) in roots of resistant flax plants invaded by *Fusarium lini*. Galls may also be developed. However, no new tissues were formed around the breaks in the cortex of the corn mesocotyl in consequence of needle pricks or of root emergence. The walls of the cells bordering on the wounds merely became more or less suberized. In the corn plant parasitized by *Gibberella saubinetii* no evidence of the formation of a corky layer in advance of penetration was observed at low temperatures.

Miehe (*26*) reports that when the epidermis was stripped from the leaves of *Allium nutans* the nuclei of the epidermal cells moved through the walls into the cells next above. Schüroff (*31*) observed a similar behavior of nuclei when he stripped off pieces of the epidermis of leaves of *Iris germanica* and when he wounded the tissues by means of a cut. No such phenomena were observed to follow the wounding of the corn plant by fungus invasion, root emergence, or needle pricks.

No attempts were made to determine whether wounding or infection by the fungus influenced nuclear size. The nuclei of normal cells are frequently much lobed, accurate measurements being therefore difficult or impossible. There is some slight evidence that this lobing is more pronounced in the vicinity of wounds and lesions, and it also appears to be more or less associated with a distributed condition of the stainable cytoplasm. (Fig. 3, I and J.)

From the time of the first observation of reactions of this class to mechanical wounds and fungus invasions, attempts have been made to determine the nature of the stimulus capable of calling forth these responses. Schürhoff (*31*) holds that the formation of new tissues as a result of wounding may be reduced to a purely mechanical cause—the relief of tissue tension. Küster (*24*) thinks it "very probable that in the production of callus, wound wood, and others, the removal of the bark pressure and similar hindrances to growth are not without an influence on the formation of the wound tissue. However, there is no necessity for the assumption that the removal of the pressure effects causes the formation of the wound tissue."[9] Jost (*23*, *p. 327*) says, in referring to wound cork, that "we must not regard the destruction of the connection with neighboring cells as the *only* factor inducing this reaction, for materials from the injured cells or other changes taking place during the infliction of the injury might act as a stimulus." Miehe (*26*) does not consider the mechanical stimulus of wounding sufficient alone to account for the nuclear reactions.

The possibility of the action of a chemical stimulus of some kind is suggested in Jost's statement above. Ritter (*30*) attempted to determine whether the nuclear response which occurs when the epidermis of an onion-bulb scale is wounded is identical with chemotaxis. He found that the two phenomena, traumatotaxis and chemotaxis of the nuclei, resulted in similar appearances, but that chemotaxis occurred at a much slower rate than traumatotaxis. He concludes that "traumatotaxis is perhaps the result of an inner change not to be treated experimentally which occurs upon wounding."[9]

The present studies on the corn plant do not throw any additional light upon the nature of the stimuli here concerned. That a stimulus

[9] Translation.

of some kind results from the ruptures and wounds in the cortex and is more far-reaching than a stimulus that results merely in the suberization of the cells bordering upon the wound is shown by a comparison of A and B in Figures 9 and 10.

Certainly, as regards the wounds produced by fungal lesions, there is a chemical stimulus in addition to whatever mechanical or wound stimuli are supplied in the formation of the lesion. But how much of the nuclear reaction may be a chemotactic response to the products of the fungal metabolism and how much a traumatotactic response is impossible to determine. Chemical activity also undoubtedly accompanies the rupturing of the cortex by the emergence of adventitious roots. The visible alterations in the cytoplasm found associated with these wounds, as well as the fungal lesions, may be manifestations on the part of the protoplasm of a response to certain chemical stimuli.

SUMMARY

Gibberella saubinetii (Mont.) Sacc., which causes a seedling blight of corn (*Zea mays* L.), enters the host usually through ruptures in the cortex produced by the emergence of adventitious roots or by the pulling apart of cells in consequence of rapid growth. Fungus invasion is at first intercellular; hyphae may also grow between the cells through the middle lamellae and in the individual cell walls. Later the cells themselves become invaded. Intercellular invasion of the tissues is ordinarily two or three cells in advance of cell-wall penetration.

The fungus, in growing from cell to cell, penetrates the cell wall by means of a fine filament. It can not be conclusively demonstrated that the process of penetration is either a mechanical or a chemical one; there is some slight evidence that a chemical action may take place at the point of penetration.

Penetration of the unruptured coleorhiza usually takes place through the epidermis of the apical portion at points where pluglike structures have been formed by the deposition of material between the epidermal cells. The fungus appears to be capable of dissolving this material and thus of gaining entrance into the host.

Invasion is accompanied by an accumulation of dark-staining material in the walls and intercellular spaces of the host tissues. The area in which this accumulation occurs extends considerably beyond the limits of fungus penetration. The deeply stained material may be a product resulting from the action of enzymes secreted by the fungus either upon substances (pectinlike materials and pentosans) normally present in the cell walls of the host tissues or upon similar substances secreted into these regions by the protoplasm. In extensively invaded areas this densely stainable material has decreased somewhat in amount, perhaps having been used as food by the fungus.

Invasion may also induce a swelling of the cell walls, especially of the walls of cells near the endodermis and of those of the hypodermal layers.

The endodermis of semiresistant strains of corn may serve as a partial if not a complete barrier against fungus penetration. It is not so effective in this respect in susceptible strains.

Callosities occur frequently on the walls of cells near the endodermis and in the hypodermal layers. These structures may be manifestations of a certain type of resistance.

A traumatotactic displacement of the nucleus was observed in cells bordering lesions and wounds in the cortex of the mesocotyl produced by root emergence or by needle pricks. An alteration in the distribution and amount of the stainable portion of the cytoplasm was observed in the cells adjacent to ruptures and lesions.

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HETEROSPILUS CEPHI ROHWER, A PARASITE OF THE EUROPEAN WHEAT SAWFLY, CEPHUS PYGMAEUS (L.)[1]

By C. C. HILL, *Associate Entomologist*, and H. D. SMITH, *Assistant Entomologist*, *Division of Cereal and Forage Insects, Bureau of Entomology, United States Department of Agriculture.* [2]

INTRODUCTION

Heterospilus cephi [3] Rohwer is a major parasite of the European wheat sawfly, *Cephus pygmaeus* (Linnaeus). It was first reared from this host in 1924, by Donald T. Ries. In 1925, it was described by Rohwer,[4] and the following year reference was made to it by Ries [5] in a paper on the wheat-stem sawfly.

It is the purpose of this paper to present certain details of the morphology and life history of this parasite.

GEOGRAPHICAL DISTRIBUTION

Heterospilus cephi has been found throughout most of the western wheat areas of the State of New York, from Syracuse and the Finger Lakes westward to Lake Erie, and over a limited area in northern Pennsylvania, but has not yet been recovered south of Lycoming County, Pa. Figure 1 shows the districts of New York and Pennsylvania from which it has been recovered.

HOST

Under natural conditions *Heterospilus cephi* has been found to attack only the European wheat sawfly, *Cephus pygmaeus* (L.). However, when introduced into a large outdoor cage containing wheat infested with the black grain-stem sawfly, *Trachelus tabidus* (Fab.), it parasitized this species freely and passed through its complete normal life cycle on this host without difficulty. This would indicate its ability to adapt itself to this host even though it does not already normally parasitize it. It is possible that the different geographical ranges of these two sawflies may account for the apparent failure of *H. cephi* to attack *T. tabidus*, as the latter has a distinctly more southern range of distribution than has *C. pygmaeus*.

RATE OF PARASITISM

In Table 1 are summarized the percentages of European wheat sawflies found parasitized by *Heterospilus cephi* during the years from 1924 to 1929 in the State of New York. The counties from which material was obtained included Cayuga, Chemung, Erie, Genesee,

[1] Received for publication Apr. 3, 1931; issued October, 1931.

[2] The authors are indebted to Prof. C. R. Crosby, of Cornell University, for furnishing infested wheat stubble from various parts of New York State; to Dr. D. T. Ries for rearing much of the material during the first year of the investigations, and to Esther H. Hart, of the Bureau of Entomology, U. S. Department of Agriculture, for drawings of the adult.

[3] Order Hymenoptera, superfamily Ichneumonoidea, family Braconidae.

[4] ROHWER, S. A. FIVE BRACONID PARASITES OF THE GENUS HETEROSPILUS. Jour. Wash. Acad. Sci. 15: 177–182, illus. 1925.

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Livingston, Monroe, Niagara, Onondaga, Ontario, Orleans, Schuyler, Tompkins, Wayne, Wyoming, and Yates. The percentages of wheat stalks infested by the host are included in the table to show the relative abundance of host and parasite.

TABLE 1.—*Percentages of Cephus pygmaeus parasitized by Heterospilus cephi in the State of New York from 1924 to 1929, inclusive*

Year	Counties	Wheat stalks infested by C. pygmaeus	Host insects examined	Host insects parasitized[1] by H. cephi
	Number	*Per cent*	*Number*	*Per cent*
1924	16	31	1,959	21
1925	17	19	2,376	25
1926	15	13	544	32
1927	13	3	100	39
1928	17	3	124	9
1929	15	4	218	1

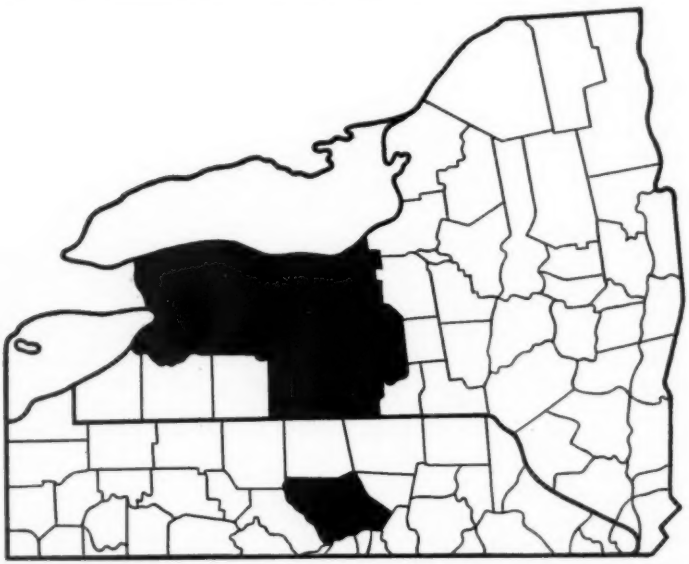

FIGURE 1.—Geographical distribution of *Heterospilus cephi*. Black area shows counties in New York and Pennsylvania where these parasites have been found

During the four years 1924 to 1927, inclusive, parasitism steadily increased from 21 to 39 per cent. During the same interval the abundance of the host decreased from 31 to 3 per cent. In the next two years parasitism dropped to 9 per cent and then to 1 per cent. It is the belief of the writers that the amount of parasitism was too small to account for the rate of decrease in abundance of the host, although it was undoubtedly a contributing factor. Furthermore, as the life history of the host is quite different from that of the parasite, the factors causing its decrease would not necessarily affect the parasite directly. For this reason a gradual increase in the rate of parasitism could be expected to occur as long as host material was

available in sufficient quantity. The sharp decrease in parasitism observed in 1928 and 1929 can be accounted for by the scarcity of host material that prevailed during the period 1927 to 1929. Such a condition would so decrease the numbers of parasites in a field that their search for hosts would become increasingly difficult.

MORPHOLOGY

ADULT

Rohwer[6] described the adult (fig. 2; fig 3, A) as follows:

Female.—Length, 2.25 mm. Length of ovipositor, 0.5 mm. Head behind the ocelli finely transversely striate; antenna 27-jointed, the joints poorly differentiated; pronotum not dentate laterally; scutum and prescutum rather coarsely

FIGURE 2.—*Heterospilus cephi*, adult female, very much enlarged

granular; notauli not foveolate, well defined; mesepisternum irregularly wrinkled dorsally with smooth, polished area ventrally; propodeum with the lateral-basal area finely granular, the lateral-dorsal carinae complete, with two diverging carinae dorsally which become obsolete laterally and posteriorly; the posterior face and dorsal surface between the diverging carinae irregularly wrinkled and with an indistinct median carina basally; first tergite sharply carinate laterally, longitudinally striate with the striae slightly irregular medianly; second tergite striato-granular; the base of the third and fourth tergites finely striate; ovipositor one-third as long as the abdomen; first intercubitus obsolescent; stigma angulate at the middle where the radius leaves it. Dark ferruginous; antennae, propodeum and first tergite piceous; wings hyaline, strongly iridescent; venation pale brown.

Male.—Length, 2.5 mm. Dorsal part of the head more feebly sculptured than the female; base of the fourth tergite feebly striate; the diverging carinae of the propodeum shorter than in the female and the median carina better defined; the posterior face of the propodeum more distinctly reticulate; antenna 31-jointed.

Type locality.—Ithaca, New York.

[6] ROHWER, S. A. Op. cit., p. 178.

It is evident, from the average dimensions of the species, that the type specimen was an abnormally small individual.

The entire length of the ovipositor sheath (fig. 3, B) is 1 mm. The distal half of the sheath is normally exserted, and this portion is sparsely setose.

EGG

The egg (fig. 4, B) is elongate, kidney-shaped, somewhat curved, and tapers from a bluntly rounded cephalic pole to a rounded caudal pole about half as wide. The average dimensions of 10 eggs were 0.647 mm. long by 0.122 mm. wide at their greatest diameter. Deviations in length showed a maximum of 0.706 mm. and a minimum of 0.590 mm.

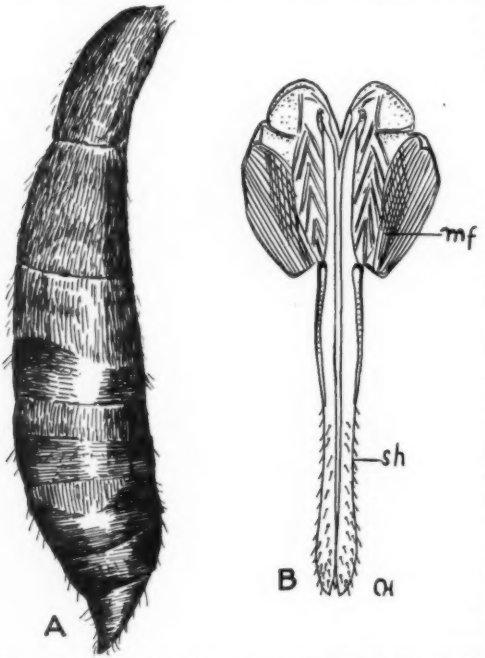

FIGURE 3.—*Heterospilus cephi:* A, Lateral aspect of abdomen of adult male, × 65; B, ovipositor of female, showing muscle fibers (*mf*) and sheath (*sh*), × 75

LARVA

FIRST INSTAR

The newly hatched larva (fig. 4, A) is circular in cross section and tapers toward each extremity, the head being broader and blunter than the caudal end. A typical larva measured 0.677 mm. in length by 0.176 mm. in width. Its general color is translucent whitish. There are 13 well-defined body segments, besides the head. Spiracles occur on the posterior part of the first thoracic segment and on the anterior part of the first eight abdominal segments. The entire body is almost entirely covered with minute spines, with the exception of the head and the median dorsal area. The head (fig. 4, E) has two conspicuous antennae 0.033 mm. in length. The length of the outer edge of the mandibles (fig. 4, D) is 0.0266 mm. From five to six teeth in comblike arrangement are along the inside edge. The labrum bears four setae, and two pairs of setae are present on each side of the mouth.

SECOND INSTAR

The second instar, in general respects, resembles the last. The body varies considerably in size according to the length of time the insect has been feeding. A minimum length of 0.90 mm. and a maxi-

mum of 2.20 mm. were found. The antennae on the head are smaller than in the first instar and measure 0.009 to 0.013 mm. in length. The mandibles (fig. 4, F) are each provided with six teeth in comblike arrangement similar to that found in the first and last instars, with a length along the outer edge of 0.037 mm.

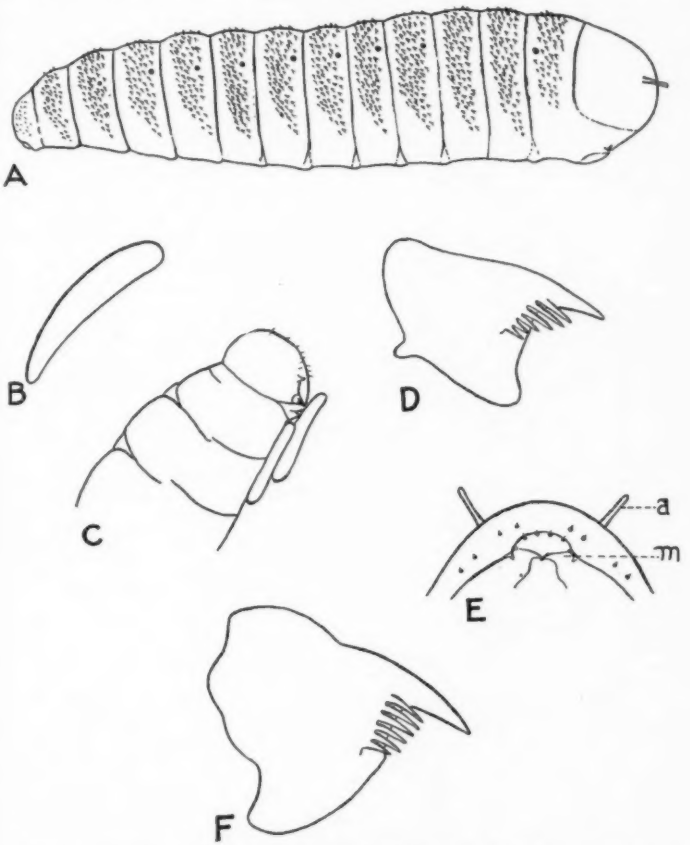

FIGURE 4.—*Heterospilus cephi:* A, Lateral aspect of first-instar larva, × 150; B, egg, × 45; C, eggs on host larva, much enlarged; D, mandible of first-instar larva, × 1,200; E, head and mouth parts of first-instar larva, × 240; *a*, antenna; *m*, mouth; F, mandible of second-instar larva, × 1,300

LAST INSTAR

The mature larva (fig. 5, A, and fig. 7, A, B) is white and tapers bluntly toward each extremity. A typical larva which had been removed from its cocoon and measured while alive had a length of 2.9 mm. and a width of 1.8 mm. There are 13 segments, besides the head. Spiracles occur on the first thoracic segment and the first eight abdominal segments. Figure 5, B, shows that the surface of the body, with the exception of the head and caudal segment, is

thickly covered with short tegumentary spines. Setae, slightly larger than the spines, are sparsely distributed on all the segments of the body, including the head. Conical, fleshy antennae 0.0166 mm. long are present on either side of the head. (Fig. 6, D.) The sensorial structures of the mouth parts (fig. 6, D) include 3 pairs of sensory spines on the area of the labrum, 6 on that of the labium, and 2 on each of the maxillary areas. Small disks (fig. 6, B, D), representing modified palpi, are found, one on each maxillary area,

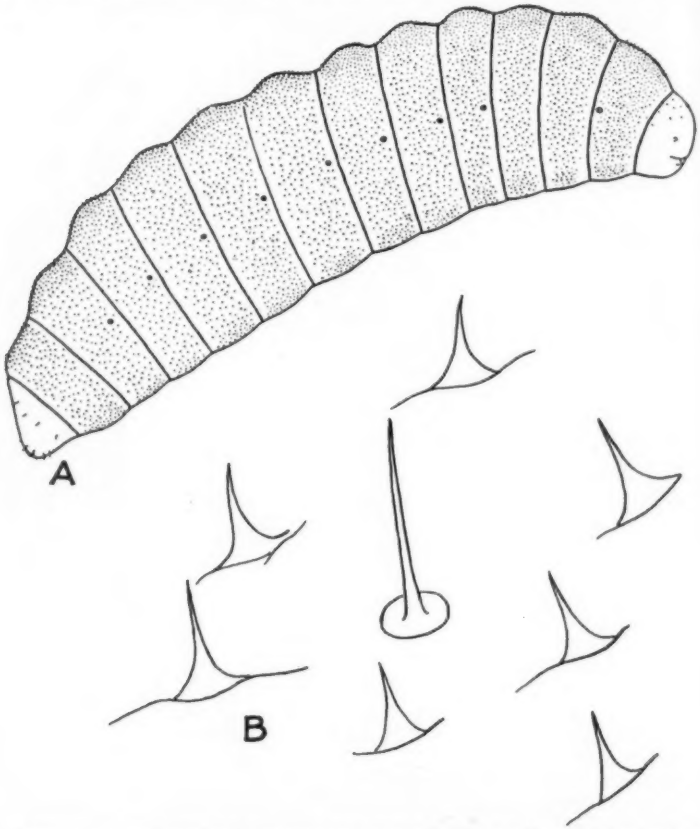

FIGURE 5.—*Heterospilus cephi:* A, Last-instar larva, × 40; B, portion of epidermis of last-instar larva showing several spines and a single seta near the center, much enlarged

and one on each side of the labium. Similar structures found on the maxillary area of the mouth parts of *Eurytoma parva* were termed "vestigial maxillary palpi" by Phillips.[7] Genieys [8] also refers to vestigial maxillary palpi on *Habrobracon brevicornis* (Vipionidae). Four pairs of minute setae, arranged in a row, are present dorsad,

[7] PHILLIPS, W. J. EURYTOMA PARVA (GIRAULT) PHILLIPS AND ITS BIOLOGY AS A PARASITE OF THE WHEAT JOINTWORM, HARMOLITA TRITICI (FITCH). Jour. Agr. Research 34: 743–758, illus. 1927.
[8] GENIEYS, P. HABROBRACON BREVICORNIS WESM. Ann. Ent. Soc. Amer. 18: 143–202, illus. 1925.

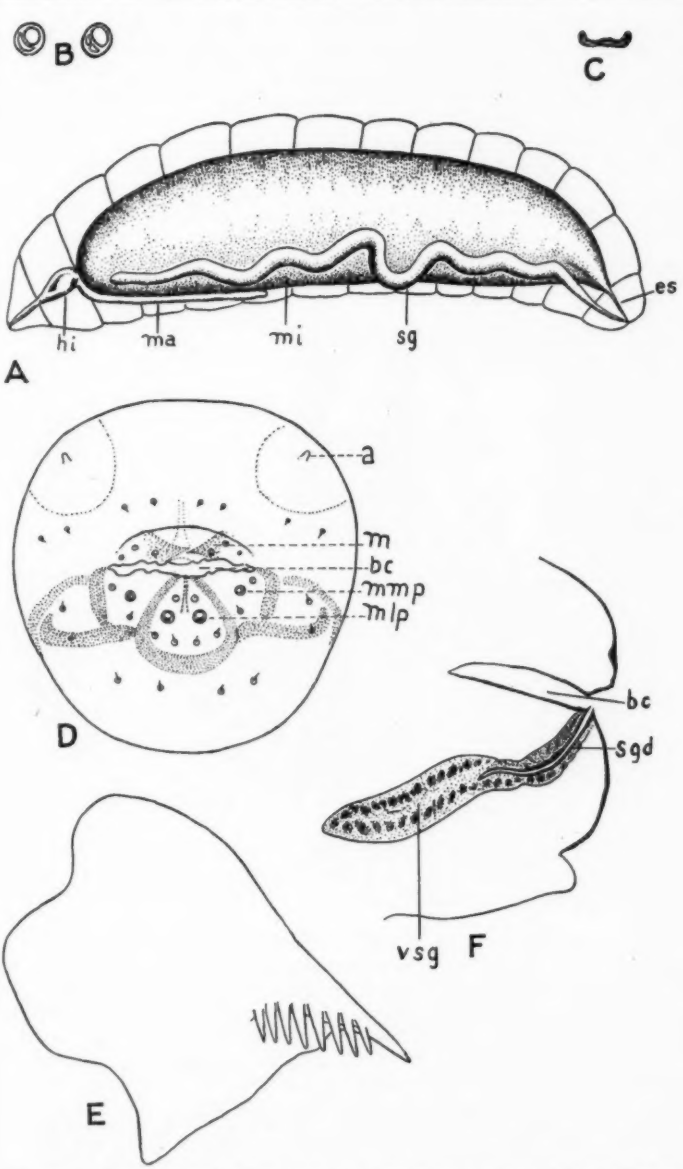

FIGURE 6.—*Heterospilus cephi*, mature larva: A, Lateral aspect of internal organs, esophagus (*es*), mid-gut (*mi*), hind-gut (*hi*), Malpighian tube (*ma*), silk glands (*sg*); B, modified palpi; C, cross section of silk-gland duct, made close to labium, ×265; D, head and mouth parts, antenna (*a*), buccal cavity (*bc*), mandible (*m*), modified labial palpus (*mlp*), and modified maxillary palpus (*mmp*); E, mandible; F, sagittal section of head of larva which has spun its cocoon, showing silk-gland duct (*sgd*), buccal cavity (*bc*), and remains of silk gland (*vsg*) after reduction in size has taken place

and two pair ventrad to mouth parts. The mandibles (fig. 6, E) are heavily chitinized, triangular in outline, with a row of eight long, narrow teeth along the inner margin. The length of the outer margin of the mandible is 0.0533 mm. It is interesting to note the similarity of structure between the mandibles of this species and those of *H. brevicornis* as described by Genieys.

INTERNAL ORGANS OF THE MATURE LARVA

The alimentary canal includes a slender esophagus extending into the anterior part of the first or prothoracic segment, a large mid-gut reaching from the prothoracic segment to the eighth abdominal segment, and a hind-gut (fig. 7, C, and fig. 6, A) with a pear-shaped

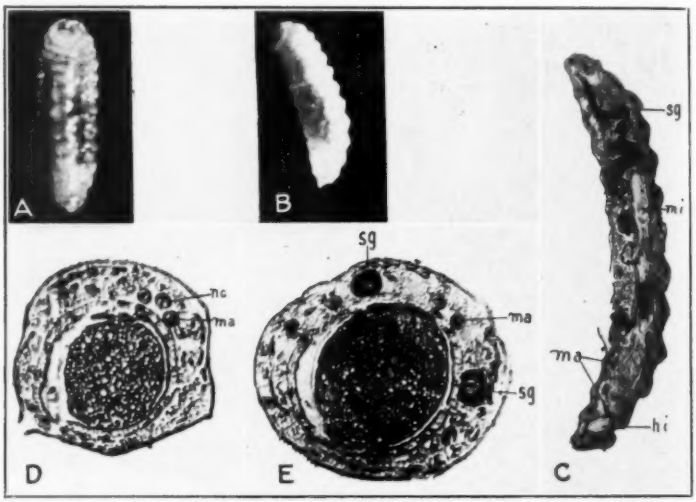

FIGURE 7.—*Heterospilus cephi*, mature larva: A, Ventral aspect; B, lateral aspect; C, sagittal section showing silk glands (*sg*), mid-gut (*mi*), hind-gut (*hi*), and parts of Malpighian tubes (*ma*), × 20; D, cross section of eighth abdominal segment before cocoon has been spun, showing Malpighian tubes (*ma*), nerve cord (*nc*), and mid-gut (photomicrograph × 40); E, cross section in region of sixth abdominal segment before cocoon has been spun, showing Malpighian tubes (*ma*), silk glands (*sg*), and mid-gut (photo-micrograph × 40)

enlargement within the ninth abdominal segment and a slender conduit within the tenth. This terminates in a transverse anal opening.

The large intestine (fig. 7, D, E, and fig. 6, A), when filled with food, occupies most of the body cavity. It remains as a blind sac until after the cocoon has been spun, when an opening is made into the hind-gut. The meconium is then voided, and the stomach contracts to about one-third its previous size and appears as a slightly distended tube continuous with the esophagus and the hind-gut.

The two Malpighian tubes (fig. 7, D, E, and fig. 6, A) arise from the anterior end of the hind-gut. They curve slightly near their point of attachment and then extend as two straight rods along the ventral side of the mid-gut, one on each side of the ventral nerve cord, until they terminate within the fifth abdominal segment.

In number and appearance these tubes are similar to those found in the larva of the vipionid *Habrobracon brevicornis*,[9] although in the latter they are considerably longer in proportion to the length of the body of the larva. They differ in number from those found on other hymenopterous larvae, the ichneumonid *Eulimneria crassifemur*[10] having four Malpighian tubes instead of two, and the Chalcidoidea, according to citations by Parker,[11] having three tubes, one of which extends caudad.

The silk glands (fig. 7, C, D, E, and fig. 6, A) consist of two stout undulating tubes extending on each side of the body cavity from the buccal region into the eighth abdominal segment. They unite close to the buccal cavity to form a single very slender chitinous duct (fig. 6, C, F) which opens at the lip of the labium where minute teeth serve as a guide for the exuded silk. In cross section the lumen of the duct in the head is compressed and somewhat crescent shaped. (Fig. 6, C.) Before pupation takes place the silk glands become reduced in size, and it has been possible to find only a small amount of glandular structure left in the region of the head. (Fig. 6, F.)

The glands differ considerably from those in the braconid *Chelonus texanus* Cress. Unpublished observations made by the senior writer on the larva of this species show the occurrence of four lateral branches instead of two. The same is true for the vipionid *Habrobracon brevicornis*,[12] and for the ichneumonid *Eulimneria crassifemur*.[13] On the other hand, according to Parker[14] the Chalcidoidea have only a single lateral branch, one on each side of the stomach, as does *Heterospilus cephi*.

COCOON

The cocoon (fig. 8, C) is cylindrical, slightly ovoid, and about 5 mm. long by 1.5 mm. wide. It is whitish and usually somewhat sprinkled with pale brown, finely granulated frass. The exit hole of the parasite is roughly oval, with a ragged edge, and is about 1.0 mm. long by 0.9 mm. wide.

The cocoons are found within the hollow center of the wheat culm where the host has been feeding and may occur almost anywhere along the stem except in the extreme base and terminal portions. Sometimes they are found in groups at different places in the stem. When more than one are present, they are usually placed end to end, with a thin partition of woven silk and frass between them.

PREPUPA

The prepupa (fig. 8, A) is white, more nearly cylindrical than the larva, and has a noticeable constriction in the region of the third thoracic and first abdominal segments. A typical prepupa measured 3.1 mm. long by 0.8 mm. wide. The eyes of the developing adult are faintly visible under the integument of the first thoracic segment.

[9] GENIEYS, P. Op. cit.
[10] THOMPSON, W. R., AND PARKER, H. L. THE MORPHOLOGY AND BIOLOGY OF EULIMNERIA CRASSIFEMUR, AN IMPORTANT PARASITE OF THE EUROPEAN CORN BORER. Jour. Agr. Research 40: 321-345, illus. 1930.
[11] PARKER, H. L. RECHERCHES SUR LES FORMES POST-EMBRYONNAIRES DES CHALCIDIENS. Ann. Soc. Ent. France 93: 261-379, illus.
[12] GENIEYS, P. Op. cit.
[13] THOMPSON, W. R., AND PARKER, H. L. Op. cit.
[14] PARKER, H. L. Op cit.

PUPA

The newly formed pupa (fig. 8, B) is nearly white, but the eyes turn dark in the course of a few days and the general coloration of the adult is assumed before eclosion takes place. Measurements of 10 pupae of each sex gave for the female an average length of 4.36 mm., with a maximum of 4.8 mm. and a minimum of 3.9 mm.; and for the male an average of 3.69 mm., with a maximum of 4.4 mm. and a minimum of 2.3 mm. The ovipositor in the female extends beyond the tip of the abdomen, and the antennae extend down each side of the body beyond the abdomen and nearly to the extremity of the ovipositor. The antennae are shorter in immature pupae, extending, in the male, just beyond the tip of the abdomen. The wing pads of both sexes reach to about the base of the second abdominal segment.

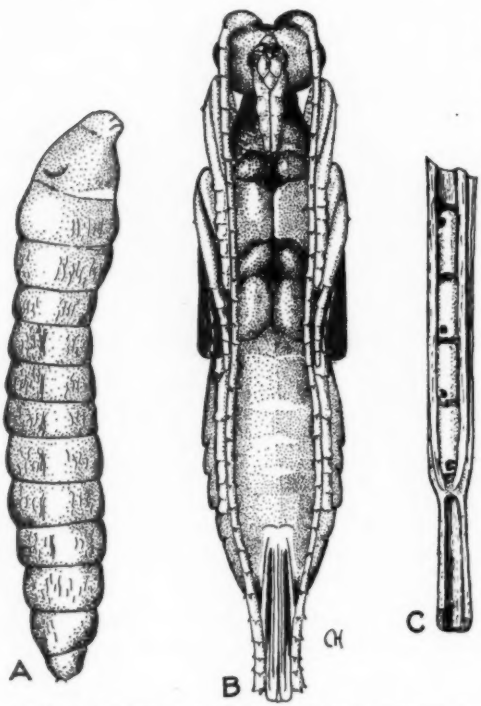

FIGURE 8.—*Heterospilus cephi:* A, prepupal stage; B, ventral aspect of pupa; C, wheat stem containing cocoons showing exit holes

LIFE HISTORY AND HABITS

SYNOPSIS

The incubation period lasts only a few days, and the early development of this parasite from the time of oviposition to the formation of the cocoon requires about three weeks. At least 10 months are then spent as a fully developed larva within the cocoon, before further transformation takes place. Three instars were observed in the larva. Pupation occurs with the advent of warm spring weather, and the prepupal and pupal periods combined occupy about one month. The adults live two or three weeks after emergence. The complete life cycle takes an entire year.

SEX RATIO

From 644 adults reared from European wheat sawfly material, collected from various parts of New York, 47 per cent were females and 53 per cent males. In the majority of cases both sexes developed on the same host.

LONGEVITY OF ADULT

In order to estimate the longevity of the adult two large vials were selected, 23 males placed in one and 19 females in the other. The air content was kept humid by the daily addition of a few drops of water, and the vials were kept tightly stoppered to prevent their drying out. The results gave for the males an average length of life of 10 days, with a maximum of 16 and a minimum of 2 days; for the females, an average of 9 days, with a maximum of 12 and a minimum of 6 days.

In another experiment the adults were kept in a humid atmosphere in individual vials. Six individuals of each sex were observed, and the results gave for the males an average length of life of 13 days, with a maximum of 18 and a minimum of 10 days; and for the females, an average of 12½ days, with a maximum of 15 and a minimum of 7 days.

In the vials in which a number of the parasites were confined together in a relatively small area, the mutual interference of the parasites in moving about may have caused more constant activity and thereby accounted for the shorter length of life than occurred when a separate vial was used for each adult. Food was not furnished in either of these experiments.

OVIPOSITION

The female usually alights rather high upon the wheat culm and then crawls up and down the stalk until a favorable place for oviposition is found, whereupon she inserts her long ovipositor through the wheat stalk until it comes in contact with the host larva.

There is evidence that from one to six eggs, and possibly more, may be laid on a host at a time. Several hosts were found bearing 1 egg each, two with 2 eggs each, one with 4 eggs, and one with 6. Where more than one egg was found, the uniform arrangement indicated that the eggs were laid at the same time by a single parasite.

Of 8 hosts examined, 5 bore eggs on the head, 1 on the underside of the thorax, 1 on the side of the fourth abdominal segment, and 1 on the anal extremity.

It is evident that the female stings the host, probably just previous to depositing the eggs, as all hosts found with eggs or larvae on their bodies are in a torpid condition and able to react only slightly to outside stimulus.

The position of the host at the time of oviposition has, in the cases observed, been in the upper part of the wheat stalk.

HABITS OF THE LARVA

The host remains in a comatose condition from the time the eggs are laid on it until death. The larva crawls freely over the body of its victim and feeds through the epidermis. Most of the host contents are finally ingested, and the head capsule together with some of the dark and shrivelled epidermis of the body is all that remains of the host after the parasite has finished feeding. Three distinct instars have been observed.

PREPUPAL DEVELOPMENT

With the warm days of spring, the larva begins its transformation into a pupa. The length of the prepupal period of eight individuals observed under experimental conditions ranged from 8 to 16 days, with an average of 10.62 days. The 2-hour mean temperature of the atmosphere outside the cage during the experiment was 64° F.

PUPAL DEVELOPMENT

The pupal period lasts from 19 to 22 days, according to two experiments made to determine its length.

In the first, which was conducted in the spring of 1926, six male and six female pupae were used. The results show, for the females, an average of 19.17 days, with a maximum of 21 and a minimum of 17 days; for the males, an average of 18 days, with a maximum of 20 and a minimum of 15 days.

In the second experiment, conducted during the spring of 1927, 6 males and 10 females were used, and thermographic records were kept of the atmosphere surrounding the containers. The mean temperature based on readings made every two hours during this experiment was 64° F. Although the days were warm, the nights were quite cool, which accounted for the rather low mean temperature. The results showed for the females an average of 22.3 days, with a maximum of 26 and a minimum of 20 days; for the males, an average of 20.5 days, with a maximum of 23 and a minimum of 17 days.

The parasitic larvae were removed from their cocoons and confined in individual, small, shell vials, the moisture content of which was kept close to the saturation point. Close daily observations were kept of their time of pupation up to eclosion.

In both experiments the pupal period was from one to two days shorter for the male than for the female. This fact helps to account for the earlier appearance of the males. Males were also observed to emerge several days earlier than females from wheat stalks stored in the laboratory rooms.

NUMBER OF PARASITES PER HOST

From an examination of 747 wheat stems it was found that from 1 to 10 cocoons occurred in a single stem, the average number being 3 per stem. As only in rare instances does more than a single host occur in a wheat stem, three is a close estimate of the average number of these parasites capable of developing on a single host. The 10 individuals found in a stem may have developed from more than one host.

SEASONAL HISTORY

There is one generation of the parasite a year, which is coincident with the single generation of the host. The adults emerge somewhat later than do the adults of the host and may be found in the fields in the last part of June and throughout most of July. The feeding period of the individual parasite is about three weeks, at the end of which time the parasite spins its cocoon and remains quiescent, in the mature larval stage, within its silken case until the following spring, when pupation occurs.

SUMMARY

Heterospilus cephi is a major parasite of the European wheat sawfly, *Cephus pygmaeus* (L.).

So far as is known at present, its geographical distribution covers most of the western wheat-growing area of the State of New York and a limited area in northern Pennsylvania.

The percentage of parasitism increased from 21 per cent in 1924 to 39 per cent in 1927, with a coincident decrease in abundance of the

host from 31 to 3 per cent. In 1928 there was a sharp decrease in parasitism to 9 per cent, and in 1929 to 1 per cent.

There is one generation a year. Oviposition occurs during the last part of June and in early July while the host larvae are feeding within the wheat stems. The period of incubation and feeding occupies about three weeks; about 10 months are then spent in the mature larval stage within the silken cocoon. With the advent of warm days in the spring, pupation takes place, and about a month is spent in the prepupal and pupal stages. The length of life of the adult is from 1 to 2 weeks.

The female inserts her ovipositor into the wheat culm and lays from one to six eggs on the surface of the host larva feeding inside the stem. The parasitic larva remains on the surface of the host larva and feeds through the epidermis of the host. It passes through three distinct instars, and spins a whitish silken cocoon within the hollow of the wheat stem. An average of three parasites were found to mature on a single host. The sex ratio showed 47 per cent of the adults to be females.

GERMICIDAL EFFICIENCY OF MIXTURES OF PHENOLS WITH SODIUM HYDROXIDE, WITH GLYCERIN, AND WITH ETHYL ALCOHOL[1]

By F. W. TILLEY, *Senior Bacteriologist*, and J. M. SCHAFFER, *Chemist, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

In a previous paper(*5*)[2] the writers called attention to the influence of the soaps of coconut oil, castor oil, and linseed oil upon the germicidal efficiency of various phenols. The present paper reports the results of experiments designed to show the influence of sodium hydroxide, of glycerin, and of ethyl alcohol upon the germicidal efficiency of phenols. These substances are commonly used as solvents, yet comparatively little work has been done to determine their effect upon the germicidal power of the phenols and, in addition, the results reported in the literature are not always in agreement.

Hailer (*1*) found that the addition of sodium hydroxide to cresol caused a diminution of germicidal efficiency, which progressed parallel with increasing neutralization of the cresol. He did not report the effect of excess alkali. Uhlenhuth and Hailer (*7*), however, found that alkaline solutions of cresol were superior to neutral solutions for the disinfection of tuberculous sputum, but attributed this superiority to the solvent action of the alkali which facilitated penetration. In the experiments of Krönig and Paul (*2*) ethyl alcohol lowered the germicidal activity of phenol whereas Morgan and Cooper (*4*), found that ethyl alcohol increased the germicidal activity of phenol. Von Wunschheim (*9*) reported that solutions in glycerin of phenol, cresol, or thymol have very little germicidal value. He also stated that the addition of water to a glycerin solution of phenol increased the germicidal efficiency. Leonard and Feirer (*3*) have reported similar results with hexylresorcinol in glycerin.

EXPERIMENTAL PROCEDURE

The phenols used in this work and their solidification points are as follows:

	°C.		°C.
Phenol	40. 7	Orthophenylphenol	56. 5
Orthocresol	30. 4	Thymol	49. 3
Paracresol	33. 0	N-Hexylresorcinol	64. 0

Metacresol also was used. Its solidification point was undetermined, but its boiling point was 202.1°–202.2° C.

Bacteriological tests were made by the Rideal-Walker technic, modified as follows: Instead of R-W standard broth, beef-infusion broth adjusted to pH 7.2 was used as the culture medium for *Staphylococcus aureus*, whereas the unadjusted culture medium of Wright (*8*), made with Armour's peptone, was used for *Eberthella typhi;* instead of a dropping pipette, a 1 c. c. pipette graduated into tenths

[1] Received for publication Mar. 28, 1931; issued October, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 617.

was used for measuring the one-half of a cubic centimeter of culture required for the test; the medication temperature was 25° C. instead of 15° to 18°; dilutions were made up in sterile Erlenmeyer flasks instead of in cylinders; fixed amounts of the stock solution of disinfectant were added to the varying amounts of water, glycerin, or alcohol required; transfers of stock solution, water, etc., were made with sterile standardized pipettes; finally, no phenol coefficients were deduced, since it was intended that germicidal efficiency should be measured by direct comparison of the concentrations required to kill the test organisms in the same length of time.

RESULTS

Table 1 shows the results of experiments with various solutions containing phenols with and without sodium hydroxide. Metacresol and paracresol were also used in the experiments, but since the results obtained were practically the same as those for orthocresol they are not included in the table.

TABLE 1.—*Influence of sodium hydroxide upon the germicidal efficiency of phenols* [a] *against Staphylococcus aureus and Eberthella typhi*

Disinfectant	Dilution	NaOH expressed as percentage of that theoretically required for complete neutralization of phenol	Results after indicated period of exposure (minutes) [b]						Test organism
			2½	5	7½	10	12½	15	
		Per cent [c]							
Phenol	1–70	0	+	+	+	−	−	−	S. aureus.
	1–75	0	+	+	+	+	+	+	
	1–40	50	+	+	−	−	−	−	
	1–50	50	+	+	+	+	+	+	
	1–10	100	+	+	+	−	−	−	
	1–20	100	+	+	+	+	+	+	
	1–40	200	+	−	−	−	−	−	
	1–50	200	+	+	−	−	−	−	
	1–60	200	+	+	+	−	−	−	
	1–100	0	+	+	−	−	−	−	E. typhi.
	1–105	0	+	+	+	−	−	−	
	1–60	100	+	+	+	−	−	−	
	1–70	100	+	+	+	+	−	−	
	1–80	100	+	+	+	+	+	+	
Orthocresol [d]	1–160	0	+	+	−	−	−	−	S. aureus.
	1–180	0	+	+	+	+	−	−	
	1–100	50	+	−	−	−	−	−	
	1–120	50	+	+	+	+	+	−	
	1–15	100	+	−	−	−	−	−	
	1–20	100	+	+	+	+	+	−	
	1–10	110	+	−	−	−	−	−	
	1–15	110	+	+	+	+	+	+	
	1–10	120	+	−	−	−	−	−	
	1–15	120	+	+	+	−	−	−	
	1–20	120	+	+	+	+	+	+	
	1–40	200	+	+	−	−	−	−	
	1–60	200	+	+	+	+	−	−	
	1–220	0	+	+	−	−	−	−	E. typhi.
	1–240	0	+	+	+	+	−	−	
	1–80	100	+	+	−	−	−	−	
	1–100	100	+	+	+	−	−	−	
	1–120	100	+	+	+	+	−	−	

[a] Experiments conducted at 25° C.

[b] Growth (+); no growth (−).

[c] 100=percentage of NaOH theoretically required for complete neutralization of the phenol. The amounts of NaOH theoretically required for complete neutralization of 1 gm. of each of the phenols are as follows: 0.42 gm. for phenol, 0.37 gm. for cresol, 0.25 gm. for orthophenylphenol, and 0.27 gm. for thymol.

[d] Metacresol and paracresol also used; results practically the same as for orthocresol.

TABLE 1.—*Influence of sodium hydroxid upon the germicidal efficiency of phenols against Staphylococcus aureus and Eberthella typhi*—Continued

Disinfectant	Dilution	NaOH expressed as percentage of that theoretically required for complete neutralization of phenol	Results after indicated period of exposure (minutes)						Test organism
			2½	5	7½	10	12½	15	
		Per cent							
Orthophenylphenol	1-4,000	[a] 0	+	+	−	−	−	−	S. aureus.
	1-5,000	[a] 0	+	+	+	+	+	+	
	1-1,500	50	−	−	−	−	−	−	
	1-1,750	50	+	+	+	−	−	−	
	1-2,000	50	+	+	+	+	+	+	
	1-1,500	100	+	−	−	−	−	−	
	1-1,750	100	+	+	+	+	+	−	
	1-5,000	[a] 0	+	−	−	−	−	−	E. typhi.
	1-6,000	[a] 0	+	+	+	+	+	+	
	1-3,000	100	+	+	+	−	−	−	
	1-4,000	100	+	+	+	+	+	+	
Thymol	1-1,500	0	+	−	−	−	−	−	S. aureus.
	1-1,600	0	+	+	−	−	−	−	
	1-1,700	0	+	+	+	+	−	−	
	1-1,500	100	+	+	−	−	−	−	
	1-1,600	100	+	+	+	+	−	−	
	1-1,700	100	+	+	+	+	+	+	
	1-600	200	+	+	+	+	+	+	
	1-2,500	0	+	+	−	−	−	−	E. typhi.
	1-2,750	0	+	+	+	+	+	+	
	1-2,500	100	+	−	−	−	−	−	
	1-2,750	100	+	+	+	−	−	−	
	1-3,000	100	+	+	+	+	+	+	
	1-2,000	200	+	+	−	−	−	−	
	1-2,500	200	+	+	+	+	−	−	

[a] Dissolved in 8 parts of coconut-oil soap.

A study of Table 1 shows that the germicidal efficiency of phenol and orthocresol decreased coincidently with an increase in the percentage of sodium hydroxide, until the percentage of sodium hydroxide equaled or slightly exceeded that required to neutralize the phenol. When, however, the percentage of sodium hydroxide was greatly in excess of that required to neutralize, and when at the same time the dilution of either of these phenols was low, the germicidal efficiency tended to increase. This was due, for the most part, to the free sodium hydroxide, since the concentration then approximated that shown to be effective against the test organism used (*6*).

In the tests with thymol the addition of sodium hydroxide caused little or no decrease of germicidal efficiency until the percentage of sodium hydroxide exceeded that required to neutralize the thymol. Beyond that point addition of sodium hydroxide was accompanied by diminished germicidal efficiency.

The differences in the results obtained with the lower phenols, on the one hand, and with thymol, on the other, are probably due to differences in the degree of hydrolysis of the sodium phenates. The lower phenols were used for the most part in dilutions of less than 1 to 100, whereas the thymol was regularly used in dilutions 10 to 50 times greater. It is well known that dilution increases hydrolysis of the sodium salts of the phenols. It is likewise known that sodium hydroxide tends to repress hydrolysis. In the experiments here recorded the sodium phenates of the lower phenols probably under-

went very little hydrolysis, whereas the dissociation in the highly diluted sodium thymolate solutions was much more nearly complete, until the sodium hydroxide reached a concentration capable of exerting a repressing effect. In making this statement the writers assume that the germicidal power of the unhydrolyzed sodium phenates is distinctly lower than that of the hydrolyzed compounds.

With each of the phenols except orthophenylphenol it was possible to compare a simple aqueous solution with a similar solution containing sodium hydroxide. In the case of orthophenylphenol the results with and without sodium hydroxide are not strictly comparable, because soap was used in the solutions that contained no sodium hydroxide. This was necessary to dissolve the orthophenylphenol. The comparison, therefore, is essentially one between solutions of orthophenylphenol with soap in one instance and with sodium hydroxide in the other. The results indicate that the solutions with soap were superior.

The results shown in Table 2 indicate that glycerin acted to depress the germicidal efficiency of phenols and also suggest that the extent of this depression was greater with the higher than with the lower phenols.

TABLE 2.—*Influence of glycerin upon the germicidal efficiency of phenols*[a] *against Staphylococcus aureus and Eberthella typhi*

Disinfectant	Dilution	Glycerin	Results after indicated period of exposure (minutes)[b]						Test organism
			2½	5	7½	10	12½	15	
		Per cent[c]							
Phenol	1–70	0	+	+	+	−	−	−	S. aureus.
	1–40	30	+	−	−	−	−	−	
	1–50	30	+	+	+	−	−	−	
	1–60	30	+	+	+	+	+	+	
	1–100	0	+	+	−	−	−	−	E. typhi.
	1–105	0	+	+	+	−	−	−	
	1–90	30	+	+	−	−	−	−	
	1–95	30	+	+	+	−	−	−	
	1–95	15	+	−	−	−	−	−	
	1–100	15	+	+	−	−	−	−	
Orthocresol	1–160	0	+	+	−	−	−	−	S. aureus.
	1–100	30	+	−	−	−	−	−	
	1–110	30	+	+	+	−	−	−	
	1–120	30	+	+	+	+	+	+	
	1–100	40	+	+	+	+	+	−	E. typhi.
	1–220	0	+	+	+	−	−	−	
	1–180	30	+	−	−	−	−	−	
	1–200	30	+	+	+	+	−	−	
Orthophenylphenol	1–4,000	[d]0	+	+	−	−	−	−	S. aureus.
	1–5,000	[d]0	+	+	+	+	+	+	
	1–800	30	+	−	−	−	−	−	
	1–1,000	30	+	+	+	+	+	+	
	1–5,000	[d]0	+	−	−	−	−	−	E. typhi.
	1–6,000	[d]0	+	+	+	+	+	+	
	1–1,400	30	+	−	−	−	−	−	
	1–1,600	30	+	+	+	+	+	+	
Hexylresorcinol	1–5,000	0	+	+	−	−	−	−	S. aureus.
	1–6,000	0	+	+	+	+	+	−	
	1–2,000	30	+	−	−	−	−	−	
	1–3,000	30	+	+	+	+	+	−	
	1–4,000	15	+	+	+	+	−	−	
	1–14,000	0	+	+	−	−	−	−	E. typhi.
	1–16,000	0	+	+	+	+	−	−	
	1–6,000	30	+	+	−	−	−	−	
	1–7,000	30	+	+	+	+	+	−	
	1–8,000	15	+	−	−	−	−	−	
	1–10,000	15	+	+	+	+	−	−	

[a] Experiments conducted at 25° C.
[b] Growth (+); no growth (−).
[c] By volume.
[d] Dissolved in 8 parts coconut-oil soap.

Table 3 shows the results obtained with mixtures containing phenols and ethyl alcohol. In general, the alcohol increased the germicidal efficiency of the lower phenols but decreased the efficiency of the higher phenols. It will be noted, however, that thymol was slightly more efficient against *Eberthella typhi* with 20 per cent alcohol. In most instances the concentrations of alcohol were considerably below those that are germicidal for the test organisms, but in the mixtures containing hexylresorcinol the highest concentrations of alcohol approached those that are germicidal. It would seem that in such mixtures the greater efficiency was due to the germicidal action of the alcohol.

TABLE 3.—*Influence of ethyl alcohol upon the germicidal efficiency of phenols against Staphylococcus aureus and Eberthella typhi* [a]

Disinfectant	Dilution	Alcohol	Results after indicated period of exposure (minutes) [b]						Test organism
			2½	5	7½	10	12½	15	
		Per cent [c]							
Phenol	1-70	0	+	+	+	−	−	−	S. aureus.
	1-80	10	+	+	+	+	+	−	
	1-80	20	+	+	+	−	−	−	
	1-100	30	+	−	−	−	−	−	
	1-120	30	+	+	+	−	−	−	
	1-100	0	+	+	−	−	−	−	E. typhi
	1-120	10	+	+	+	−	−	−	
	1-160	20	+	−	−	−	−	−	
	1-180	20	+	+	−	−	−	−	
	1-200	20	+	+	+	−	−	−	
Paracresol	1-240	0	+	+	+	−	−	−	
	1-320	20	+	+	−	−	−	−	
	1-360	20	+	+	+	−	−	−	
	1-400	20	+	+	+	+	−	−	
None		20	+	+	+	+	+	[d] +	
Thymol	1-1,600	0	+	+	−	−	−	−	S. aureus
	1-800	30	+	−	−	−	−	−	
	1-1,000	30	+	+	+	−	−	−	
	1-1,200	30	+	+	+	+	+	+	
	1-2,500	0	+	+	−	−	−	−	E. typhi.
	1-2,500	10	+	+	−	−	−	−	
	1-2,400	20	+	+	−	−	−	−	
Orthophenylphenol	1-4,000	* 0	+	+	−	−	−	−	S. aureus.
	1-5,000	* 0	+	+	+	+	+	+	
	1-1,000	30	+	+	+	−	−	−	
	1-2,000	30	+	+	+	+	+	+	
	1-5,000	* 0	+	−	−	−	−	−	E. typhi.
	1-6,000	* 0	+	+	+	+	+	+	
	1-1,000	20	−	−	−	−	−	−	
	1-2,000	20	+	+	+	+	−	−	
Hexylresorcinol	1-5,000	0	+	+	−	−	−	−	S. aureus.
	1-6,000	0	+	+	+	+	+	−	
	1-5,000	10	+	−	−	−	−	−	
	1-6,000	10	+	+	+	+	−	−	
	1-4,000	20	+	−	−	−	−	−	
	1-5,000	20	+	+	+	+	+	+	
	1-2,000	30	+	−	−	−	−	−	
	1-3,000	30	+	+	+	+	−	−	
	1-3,000	40	+	+	−	−	−	−	
	1-4,000	40	+	+	+	−	−	−	
	1-5,000	40	+	+	+	−	−	−	
	1-14,000	0	+	+	−	−	−	−	E. typhi.
	1-16,000	0	+	+	+	+	−	−	
	1-12,000	10	+	−	−	−	−	−	
	1-14,000	10	+	+	+	−	−	−	
	1-16,000	10	+	+	+	+	+	+	
	1-10,000	20	+	+	−	−	−	−	
	1-12,000	20	+	+	+	+	+	−	
	1-12,000	30	+	+	−	−	−	−	
	1-14,000	30	+	+	−	−	−	−	
	1-16,000	30	+	+	+	−	−	−	

[a] Experiments conducted at 25° C.
[b] Growth (+); no growth (−).
[c] By volume.
[d] Growth after 1 hour.
[e] Dissolved in 8 parts coconut-oil soap.

Results shown in Table 4 indicate that soaps, in the proportions used, tend to counteract the depressing action exerted by glycerin or alcohol upon the germicidal efficiency of the phenols. This effect of the soap was somewhat greater in the presence of alcohol than in the presence of glycerin.

TABLE 4.—*Influence of soaps upon the germicidal efficiency of mixtures containing phenols with glycerin or with ethyl alcohol* [a] *against Staphylococcus aureus and Eberthella typhi*

Disinfectant	Glycerin or alcohol	Soap	Dilution of disinfectant	Results after indicated period of exposure (minutes) [b]						Test organism
				2½	5	7½	10	12½	15	
	Per cent [c]									
Orthophenylphenol	Glycerin, 30	None	1-800	+	−	−	−	−	−	*S. aureus.*
			1-1,000	+	+	+	+	+	+	
		Coconut-oil [d]	1-2,000	+	+	+	−	−	−	
			1-3,000	+	+	+	+	+	+	
		None	1-1,400	+	−	−	−	−	−	*E. typhi.*
			1-1,600	+	+	+	+	+	+	
		Coconut-oil [d]	1-2,000	+	−	−	−	−	−	
			1-3,000	+	+	+	+	+	−	
	Alcohol, 30	None	1-1,000	+	+	+	−	−	−	*S. aureus.*
			1-2,000	+	+	+	+	+	+	
		Coconut-oil [d]	1-3,000	+	−	−	−	−	−	
			1-4,000	+	+	+	−	−	−	
	Alcohol, 20	None	1-2,000	+	+	+	+	−	−	*E. typhi.*
			1-4,000	+	+	−	−	−	−	
		Coconut-oil [d]	1-5,000	+	+	−	−	−	−	
			1-6,000	+	+	+	−	−	−	
	Glycerin, 30	None	1-2,000	+	−	−	−	−	−	*S. aureus*
			1-3,000	+	+	+	+	+	−	
		Castor-oil [e]	1-3,000	+	+	−	−	−	−	
			1-4,000	+	+	+	+	+	+	
		Castor-oil [d]	1-2,000	+	−	−	−	−	−	
			1-3,000	+	+	+	+	−	−	
		None	1-6,000	+	+	−	−	−	−	*E. typhi.*
			1-8,000	+	+	+	+	+	+	
		Castor-oil [e]	1-6,000	+	+	−	−	−	−	
			1-8,000	+	+	+	+	+	+	
		Castor-oil [d]	1-6,000	+	−	−	−	−	−	
			1-8,000	+	+	+	+	−	−	
Hexylresorcinol	Alcohol, 30	None	1-2,000	+	+	−	−	−	−	*S. aureus.*
			1-3,000	+	+	+	+	−	−	
		Castor-oil [e]	1-5,000	+	+	−	−	−	−	
			1-6,000	+	+	+	+	−	−	
		Castor-oil [d]	1-4,000	+	+	−	−	−	−	
			1-5,000	+	+	+	−	−	−	
			1-6,000	+	+	+	+	−	−	
	Alcohol, 20	None	1-10,000	+	+	−	−	−	−	*E. typhi.*
			1-12,000	+	+	+	+	+	−	
		Castor-oil [e]	1-10,000	+	+	+	−	−	−	
			1-12,000	+	+	+	+	+	+	
		Castor-oil [d]	1-10,000	+	−	−	−	−	−	
			1-12,000	+	+	+	−	−	−	
			1-14,000	+	+	+	+	+	−	

[a] Experiments conducted at 25° C.
[b] Growth (+); no growth (−).
[c] By volume.
[d] Two parts of soap to one of disinfectant.
[e] One part of soap to two of disinfectant.

SUMMARY

Bacteriological tests were made with phenol, orthocresol, paracresol, thymol, orthophenylphenol, or hexylresorcinol in solutions containing sodium hydroxide, glycerin, ethyl alcohol, or soaps with glycerin or alcohol, against *Eberthella typhi* and *Staphylococcus aureus*.

The addition, to solutions of phenol or cresol, of sodium hydroxide until it equaled or slightly exceeded the amount required for neutralization, resulted in progressive diminution of germicidal efficiency. Large excess of sodium hydroxide tended toward an increase in

germicidal power of the mixture, because of the action of the free sodium hydroxide. Addition of sodium hydroxide to solutions of thymol caused very little decrease of efficiency until the amount of sodium hydroxide exceeded that required completely to neutralize the thymol. The addition of a large excess of sodium hydroxide caused a decided loss in efficiency.

Glycerin acted to depress the germicidal efficiency of all the phenols tested, and the depression increased with increasing concentration of glycerin. Alcoholic solutions of the lower phenols were more efficient than aqueous solutions, but in most instances alcohol decreased the efficiency of the higher phenols. Soaps tended to counteract the depression of germicidal efficiency exerted by glycerin or ethyl alcohol.

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71917—31——4

STUDIES OF LIGNIN IN WHEAT STRAW WITH REFERENCE TO LODGING[1]

By MAX PHILLIPS, *Senior Chemist*, JEHIEL DAVIDSON, *Associate Chemist*, and H. D. WEIHE,[2] *Junior Chemist, Chemical and Technological Research, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

The significance of lodging, the lying over of cereal plants at a period when their vegetative growth is nearly complete, and the conditions accompanying it have been discussed in a previous communication (*4*).[2]

REVIEW OF LITERATURE

The causes of lodging have been a matter of controversy among agricultural chemists for many years. Davy (*5, p. 51*) thought that silica lends support to the grasses and to other plants of the "feeble vegetable tribes." Liebig (*15, p. 167–168*) believed that lodging is due to a deficiency of silica in the stalk of the plant. He attributed the strength of the culms of the grass family to their potassium silicate content. This view was supported by Kühn (*14, p. 13–14*). Knop (*12*), Kohl (*13, p. 202–309*), and Swiecicki (*21*). The importance of silica, however, began to be questioned as a result of the work of Sachs (*19, p. 150*), Pierre (*18*), Jodin (*10*), and Mayer (*16, p. 280*). On the other hand, recently Douglas (*7*) considered a low silica content as one of the many causes of lodging in sugarcane. Headden (*8, p. 69*) and Davidson and LeClerc (*3*) found that applications of sodium nitrate, which frequently cause lodging, depressed appreciably the silica content of wheat straw. Davidson and LeClerc found that the ash content was also depressed under these conditions.

According to Sachs (*19, p. 150*) and Welton (*22*), lodging may be attributed to several causes, among which is the deficiency of lignin in the culms of the plants.

Mehta (*17*) found that whenever the "true cellulose" (as determined by a special method developed by this investigator) of wheat straw was low, there was a marked tendency to lodging.

Because there appeared to be no general agreement among investigators as to the probable cause or causes of lodging, the writers undertook an investigation of this problem, having in mind particularly the significance of the lignin content of the stalks as a contributory cause.

EXPERIMENTAL PROCEDURE

The experiments described in this paper were conducted on wheat grown during the summers of 1929 and 1930 on the Department of Agriculture Experiment Farm at Rosslyn, Va. In 1929, two wheat

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[2] Reference is made by number (italic) to Literature Cited, p. 625.

plots, about one-fiftieth acre each, were selected in the same field. One plot received no fertilizer treatment and served as a control; the other received sodium nitrate early in the spring at the rate of 600 pounds per acre, obviously a high application for wheat. The wheat on the fertilized plot lodged considerably, whereas that on the control plot remained erect until harvested. Samples were cut from the two plots at weekly intervals from the time the plants were approximately 15 inches in height up to maturity. The first samples were taken on April 30, and the last on June 25. In the early stages the entire plant was analyzed, but later, beginning with the heading time (May 21), the heads were removed and the stalks and heads were analyzed separately. In the last stages, close to maturity, the heads were separated into grain and chaff, and only the latter was analyzed.

All samples were air-dried and ground in a mill, and the total lignin, methoxyl, cellulose, silica, ash, and nitrogen were determined. The loss on drying in an oven at 105° C. of the air-dry material was determined, and all the results were calculated on the oven-dry basis and are thus reported in the tables. The percentage of methoxyl was determined by the Kirpal and Bühn (*11*) modification of the Zeisel (*9, p. 144*) method. The lignin was determined by the Dore (*6*) modification of the method of Willstätter and Zechmeister (*23*). The plant material was in every case extracted with a 1:1 alcohol-benzene solution before being subjected to hydrolysis with the fuming hydrochloric acid for the determination of lignin. The percentage of cellulose was determined by the Sieber and Walter (*20*) modification of the Cross and Bevan method. Instead of Gooch crucibles, prepared as recommended by Sieber and Walter, Jena sintered glass crucibles were used. The terms "lignin" and "cellulose" as used in this paper refer only to the materials as defined by the analytical methods employed.

RESULTS

LIGNIN, METHOXYL, AND CELLULOSE

It will be observed from the data in Table 1 that the methoxyl and lignin content of the stalks from both plots tended gradually to increase with the age of the plant. The increase in the percentage of methoxyl in the stalks may undoubtedly be attributed to the progressive lignification of the plant. In fact, the percentage of methoxyl in the stalks may be taken as an index of its state of lignification.

A comparison of the data on the lignin content shows that in every case there was a higher percentage of lignin in the lodged stalks from the fertilized plot than in the unlodged stalks from the control. There was a similar increase in the percentage of methoxyl in the stalks from the fertilized plot as compared with those from the control.

In the earlier stages of growth the percentage of methoxyl in the lignin from the lodged plants grown on the fertilized plot was considerably greater than that from the plants grown on the control plot. In the later stages of growth, however, the methoxyl content of the lignin was of the same general order of magnitude in both cases.

TABLE 1.—*Percentage content of lignin, methoxyl, cellulose, nitrogen, ash, and silica in, and nitrate tests on, stalks of plants from fertilized and from unfertilized plots*

[All results are expressed on moisture-free basis]

Date harvested	Methoxyl		Lignin		Methoxyl in lignin		Cellulose (method of Cross and Bevan)	
	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)
1929								
Apr. 30	1.10	1.32	7.02	9.54	6.41	8.01	31.7	34.9
May 7	1.30	1.61	8.46	9.20	8.37	8.54	33.0	35.0
May 14	1.59	1.92	8.82	10.58	----	12.21	34.7	39.4
May 21[c]	1.96	2.53	10.80	13.41	11.43	13.04	38.0	42.0
May 28[c]	2.27	2.40	10.03	11.80	13.62	11.80	37.6	41.2
June 4[c]	2.47	2.70	11.30	13.26	12.96	12.24	39.4	41.1
June 11[c]	2.72	2.69	12.84	13.86	12.86	11.05	43.0	40.8
June 18[c]	2.99	2.99	13.72	15.67	9.86	9.81	48.6	43.6
June 25[c]	3.04	3.17	15.47	16.72	10.20	9.75	49.6	43.7

Date harvested	Total nitrogen		Ash		Silica		Nitrates	
	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)[a]	Fertilized plot (lodged)[b]
1929								
Apr. 30	1.68	2.76	8.50	9.06	2.84	2.28	–	++
May 7	1.27	----	6.62	8.26	2.30	1.76	–	++
May 14	1.17	2.02	----	8.13	----	2.07	–	++
May 21[c]	1.01	1.93	6.83	8.09	2.89	2.46	–	++
May 28[c]	.82	1.70	7.28	8.36	2.66	2.70	–	++
June 4[c]	.70	1.72	6.69	8.12	3.09	2.61	–	++
June 11[c]	.64	1.50	7.34	7.16	3.78	2.57	–	++
June 18[c]	.50	1.59	7.09	6.80	4.21	2.55	–	++
June 25[c]	.46	1.21	6.40	5.39	4.38	2.68	–	+

[a] –=absent. [b] +=present. [c] Heads removed and analyzed separately.

With the exception of plants in the very last stages of growth, the percentage of cellulose, as estimated by the method of Cross and Bevan, in the lodged plant material from the fertilized plot was higher than that from the control plot. These data when taken in conjunction with the data on the lignin content are interesting, as they indicate that the higher percentage of lignin in the lodged plant material from the fertilized plot was not accompanied by a lower percentage of cellulose (except in the very last stages of growth), that is, the lignin did not increase at the expense of the cellulose.

These results are in distinct opposition to the generally accepted views of Sachs (*19*) and Welton (*22*) that lodging is caused by a deficiency of lignin.

In Table 2 are recorded the analytical data on the heads and the chaff from the wheat plants grown on the fertilized and the unfertilized plot. The whole heads of the plants sampled up to and including June 11 were ground and analyzed. The heads collected subsequently, however, were separated into grain and chaff, and only the latter was analyzed.

TABLE 2.—*Percentage content of lignin, methoxyl, cellulose, nitrogen, ash, and silica in and nitrate tests on, heads and chaff of plants from fertilized and from unfertilized plots*

[All results expressed on moisture-free basis]

Date harvested	Part of plant	Methoxyl		Lignin		Methoxyl in lignin		Cellulose (method of Cross and Bevan)	
		Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)
1929									
May 21	Head	1.92	1.87	12.19	12.00	10.84	10.10	42.2	41.4
May 28	do	1.42	1.60	7.47	9.18	8.58	9.54	29.3	39.7
June 4	do	1.12	1.28	5.83	8.77	5.10	6.75	21.7	24.8
June 11	do	.90	.98	5.18	5.93	7.03	5.18	19.9	19.8
June 18	Chaff	2.34	1.98	10.60	10.96	11.43	12.43	43.5	39.7
June 21	do	2.22	2.05	11.33	10.50	14.14	14.10	46.1	43.0
June 25	do	2.45	2.18	12.10	13.80	13.86	12.70	45.5	42.3

Date harvested	Total nitrogen		Ash		Silica		Nitrates	
	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged)	Fertilized plot (lodged)	Unfertilized plot (not lodged) [a]	Fertilized plot (lodged) [a]
1929								
May 21	1.65	1.77	5.58	4.61	2.71	1.89	—	—
May 28	1.57	1.70	5.63	5.50	3.32	3.15	—	—
June 4	1.53	1.74	5.70	5.70	3.28	3.28	—	—
June 11	1.51	1.57	4.91	5.93	2.76	3.39	—	—
June 18			11.48	9.98	9.03	6.32	—	—
June 21			10.57	8.89	8.95	6.58	—	—
June 25	.46	.92	10.23	8.59	9.10	7.13	—	—

[a] — = absent.

The heads of the wheat plants grown on the fertilized plot (lodged), with one exception, contained a higher percentage of lignin than those of the plants from the control plot. The methoxyl content of the heads from the plants of the two plots did not differ markedly, but in all samplings except one it was slightly higher in those from the fertilized plot than in those from the control. In the chaff the difference was pronounced, but the relation was reversed, the percentage of methoxyl being higher in the material from the control plot. The data on the cellulose and on the methoxyl in the lignin from the heads are irregular, and no definite conclusions can be drawn from them. The cellulose in the chaff from the plants of the control plot (not lodged) was distinctly higher than that in the plants from the fertilized plot (lodged). In the chaff the lignin and the methoxyl in the lignin do not show any consistent tendency in their variation.

The experiment was repeated on wheat grown during 1930 to determine what effect the quantity of sodium nitrate applied, as well as the time of application, had on the lignin content of the straw. Ten small plots were laid out in the same field on the Arlington Experiment Farm. The experiments were conducted in duplicate series. In each series one plot served as a control. To two plots an early application of sodium nitrate was made at the rate of 300 and 600 pounds per acre, respectively. To two other plots in each series the sodium nitrate was applied during heading time at the same respective rates. Samples of straw from each plot were taken when the wheat was mature and ready for harvest. The analytical procedure was exactly the same as previously described. The results are presented in Table 3.

TABLE 3.—*Percentage content of lignin, methoxyl, cellulose, nitrogen, ash, and silica in, and nitrate tests on, plants from fertilized and from unfertilized plots, 1930 (all results are expressed on moisture-free basis)*

Item	Methoxyl in plant	Lignin	Methoxyl in lignin	Total nitrogen	Ash	Silica	Nitrates *
First series:							
Control plot	3.05	13.64	12.15	0.37	5.42	2.27	—
Early application of $NaNO_3$—							
300 pounds per acre	3.07	14.54	12.19	.41	4.94	2.01	—
600 pounds per acre	3.05	15.82	12.56	.44	5.97	2.51	—
Application of $NaNO_3$ at heading time—							
300 pounds per acre	3.08	14.25	11.49	.37	7.12	3.09	—
600 pounds per acre	3.20	14.77	12.35	.42	6.89	2.83	—
Second series:							
Control plot	3.19	15.60	13.95	.36	6.06	2.54	—
Early application of $NaNO_3$—							
300 pounds per acre	3.46	15.10	13.13	.38	5.45	2.23	—
600 pounds per acre	3.11	16.99	13.93	.52	5.08	2.00	—
Application of $NaNO_3$ at heading time—							
300 pounds per acre	3.24	13.18	10.48	.40	5.17	2.19	—
600 pounds per acre	3.08	15.50	12.61	.49	5.16	2.04	—

*—=absent.

Owing to a prolonged dry spell during which both the first and second applications were made, the sodium nitrate had little effect on the yield and composition of the crops of the 1930 experiment, and the dry weather also affected the lignin changes. However, in the first series the results are fully consistent and in harmony with the results of the previous year. The lignin content of the straw from the fertilized plot was in every case greater than that from the control plot. The straw from the plots that received the earlier application of sodium nitrate had a higher lignin content than the straw from plots receiving the later application. Likewise, the straw from the plots that received sodium nitrate at the rate of 600 pounds per acre was higher in lignin than the straw from the plots that received the nitrate at the lower rate of 300 pounds per acre.

In the second series of the 1930 experiments the straw from the plot that received a high application of fertilizer at the early stage also had the highest lignin content of the series. Likewise, the straw from the plots receiving the higher application of sodium nitrate was higher in lignin than the straw from the plots receiving the lower application.

On the whole, the results of the second series are irregular. This is most likely due to the lack of rainfall mentioned above. It is also possible that the irregularity of this series may be explained in part by the fact that for some unknown reason the straw from the control plot had a very high lignin content as compared with the straw from the control plot of the first series of the same year's experiment and also as compared with the lignin content of the straw from all samplings of the control plot of the 1929 experiment.

The fact that sodium nitrate, which frequently causes lodging, increased the lignin content of the straw, as clearly shown by the results of the 1929 experiment (Tables 1 and 2), would seem to indicate that if lignin is to be considered a factor, a high lignin content and not a low one is responsible for lodging. This conclusion is in accord with the recent work of Dadswell and Hawley (*2*), who found that brash specimens of Douglas fir had a higher lignin content than tough specimens. Apparently the relatively higher lignin content makes the

straw (or wood) brittle, so that it tends to break under the violent impact of winds, whereas with a normal lignin content the plants only bend and straighten out again.

NITROGEN, ASH, AND SILICA

The percentage of nitrogen was determined by the Kjeldahl-Gunning-Arnold method (*1*) when the plant material did not contain any nitrates. When nitrates were present the total-nitrogen method was used. Nitrate nitrogen was found in the stalks from the fertilized plot in all samplings of the 1929 experiment. (Table 1.) The nitrates were determined only qualitatively with diphenylamine sulphuric acid reagent. This reagent reacts with nitrites in the same way that it does with nitrates. In previous experiments,[3] however, nitrites have not been detected in plants containing nitrates.

The nitrogen was distinctly higher in the stalks and chaff and slightly higher in the heads from the fertilized plot than in those from the control plot. The heads were taken in the earliest stages of their development before their differentiation into grain and chaff. The stalks from the fertilized plot were from 1.6 to 3 times higher in nitrogen than those from the unfertilized plot. In general, the difference gradually increased with the advance in age of the plant.

The ash content was higher in the stalks from the fertilized plot than in the stalks from the control plot through June 4. (Table 1.) On June 11 the ash content of the stalks from the two plots was about the same. In the samples taken subsequently up to maturity the ash content was invariably higher in the stalks and chaff of the not-lodged plants from the control plot than in those of the lodged plants from the fertilized plot. These data corroborate the results of Davidson and LeClerc (*3*), who found that the ash content of wheat straw at maturity was depressed by applications of nitrogen carriers.

In the first two samplings the ash content of the heads of the plants from the control plot (not lodged) (Table 2) was higher than that from the heads of the plants from the fertilized plot (lodged); in the third sampling the ash content was the same, but in the last sampling the relation was reversed. This change was most likely due to the difference in the rate of filling of the kernels, as nitrogenous fertilizers frequently delay ripening. The ash content of the chaff from the control plot (not lodged) (Table 2) was in every case higher than that from the fertilized plot (lodged).

The silica content of the stalks and chaff from the fertilized plot (lodged) (Tables 1 and 2) was with only one exception distinctly lower than that of the stalks and chaff of the control plot (not lodged). These data corroborate the previous results of Headden (*8*) and Davidson and LeClerc (*3*), who found that the silica content of wheat straw was depressed by application of inorganic nitrogenous fertilizers, and also those of Swiecicki (*21*), who obtained similar results with oats. The silica content of the heads (Table 2) showed the same tendency as the ash content, the reversal in the last sampling being most likely due to the difference in the rate of ripening of the grain of the two plots.

Previous results by Davidson and LeClerc (*3*) show clearly that the depressing effect of nitrogen carriers on the silica content of straw is due to the action of the nitrogen and not to that of basic or acid radicals with which they are combined.

[3] Unpublished results.

The ash and silica contents of the straw from the plots of the 1930 experiment do not show any consistent variations, no doubt owing to the fact that the sodium nitrate was applied during the prolonged dry spell mentioned before and consequently had only a slight effect on the yield or composition of the crops. This is clearly shown by the nitrogen content of the straw from the fertilized plots.

Swiecicki (*21*) thought that his results served to support the view of Liebig (*15*), who claimed that lodging of crops is caused by a depressed silica content in the straw. Although no such definite claim is made here, the results obtained in this investigation indicate that there is a close relationship between the silica content of the cereal straws and lodging. This relationship would seem to deserve further investigation.

SUMMARY

The lignin, methoxyl, cellulose, silica, and ash content were determined in wheat stalks cut at various stages of growth from fertilized and unfertilized plots.

Wheat stalks from the plots which received sodium nitrate and where lodging had occurred in nearly all cases contained a higher percentage of methoxyl and lignin than did the stalks from the plots which received no nitrate and where no lodging had taken place. The cellulose was also greater, except in the last stages of growth, in the stalks from the plots where lodging had occurred than in the stalks from the plots where the plants did not lodge. These results disprove the idea of Sachs, who held that lodging was caused by a deficiency of lignin in the stalks.

The increase in lignin caused by an early application of sodium nitrate seemed to be directly related to the rate of application.

The application of even excessive quantities of sodium nitrate to wheat at heading time did not bring about any appreciable increase in the lignin content of the straw.

In the early stages of growth the ash content was higher in the stalks from the fertilized plot (lodged) than in those from the unfertilized plot (not lodged), but in the later stages the relation was reversed.

Sodium nitrate caused a distinct decrease in the silica content of the straw.

The results on the silica content of the straw seem to furnish new evidence in support of the original view of Liebig, who maintained that lodging was caused by a deficiency of silica in the straw.

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A STUDY OF OVA FROM THE FALLOPIAN TUBES OF DAIRY COWS, WITH A GENITAL HISTORY OF THE COWS [1]

By FRED W. MILLER, *Senior Veterinarian and Physiologist*, and W. W. SWETT, *Senior Dairy Husbandman, Bureau of Dairy Industry, United States Department of Agriculture;* and CARL G. HARTMAN and WARREN H. LEWIS, *Department of Embryology, Carnegie Institution of Washington*

INTRODUCTION

The need for information on the production and characteristics of the ovum and the conditions affecting its fertilization in the cow has been felt. To this end the department of embryology of the Carnegie Institution of Washington and the Bureau of Dairy Industry of the United States Department of Agriculture entered into a cooperative arrangement to study the ovum of the cow.[2] The cows used in the experiment were provided by the Bureau of Dairy Industry from its herd at the United States dairy experiment station at Beltsville, Md., and were slaughtered in accordance with the procedure regularly followed in studying the relation between conformation, anatomy, and milk and butterfat producing capacity. The Carnegie Embryology Laboratory was represented at the time each cow was slaughtered. The genital organs were taken to the laboratory of the Carnegie Institution in Baltimore, Md., for the purpose of locating the ovum, photographing it, and studying its anatomy and other characteristics.

A difficulty in studying the ovum of the cow is that usually only one ovum is produced at an ovulation, which ordinarily, but not always, occurs at each period of heat. Then too, the ovum is not discharged from the body at any definitely known time as is the case with the spermatozoa. If fertilization does not take place the escape of the ovum from the body is not observed because of its minute size. For these reasons the exact time of ovulation and the nature of the mature ovum have been difficult to determine. A knowledge of the time of ovulation, time of fertilization, conditions favorable for fertilization, and the characteristics of the ovum as well as the spermatozoon, is of vital importance to the biologist, the geneticist, and the breeder of dairy cattle.

REVIEW OF LITERATURE

According to Hartman's tables and bibliography of works dealing with the early development of the mammalian egg (*8*)[3] some progress has been made in the study of tubal ova of domestic animals. Tubal ova of the pig have been satisfactorily studied by Keibel (*12*), Assheton (*2*), Corner and Amsbaugh (*6*), and Heuser and Streeter (*11*). Tubal ova from the sheep were seen and described by Haus-

[1] Received for publication Apr. 14, 1931, issued October, 1931.

[2] The success attending the efforts to secure early stages of the cow ovum was due to the cooperation of the Carnegie Laboratory of Embryology, Dr. G. L. Streeter, director, and the Bureau of Dairy Industry, U. S. Department of Agriculture, through R. R. Graves, Chief of the Division of Breeding, Feeding, and Management Investigations.

[3] Reference is made by number (italic) to Literature Cited, p. 635.

mann (*10*), Bischoff (*3*), and Assheton (*1*). Bischoff (*4*) recovered several from the deer. The youngest deer ovum studied by Keibel (*13*) and Sakurai (*16*) had just reached the uterus.

EXPERIMENTAL ANIMALS

Three cows were employed. One cow furnished a 2-celled ovum; one failed to ovulate; and the other ovulated, but the ovum, which was recovered, had not been fertilized. So far as known to the writers, ova from the Fallopian tube of the dairy cow have never before been found. In this paper are presented the results of a study of two tubal ova of the dairy cow, as the beginning of our knowledge of this phase of reproduction in this animal.

GENITAL HISTORY

A review of the available records of the genital functions of the cows follows:

Cow A–11 was born February 7, 1923. Previous to this experiment, eight matings resulted in six conceptions. During the second sexual season only one heat occurred. She was bred, and she conceived at that time. During the third sexual season there were three heats at intervals of 61 and 22 days, with conception at the end of the 22-day cycle. Three heat periods, with services during each period, occurred at intervals of 45 and 20 days during the fourth sexual period. Conception followed at the end of the 20-day cycle. Only one heat occurred during each of the next two sexual seasons. The genital history of cow A–11 is given in Table 1.

TABLE 1.—*Genital history of cow No. A–11*

Dates of heats and breedings	Interval between observed heats	Service	Date of calving	Length of gestation period	Pregnancy No.
	Days			*Days*	
June 4, 1924	(?)	First.	Nov. 13, 1924	[a] 152	1
Feb. 15, 1925		First.	Nov. 23, 1925	281	2
Jan. 21, 1926	61				
Mar. 23, 1926	22				
Apr. 14, 1926		First.	Jan. 20, 1927	281	3
Apr. 19, 1927	45	First.			
June 3, 1927	20	Second.			
June 23, 1927		Third.	Mar. 20, 1928	281	4
June 2, 1928		First.	Mar. 15, 1929	286	5
Apr. 18, 1929		First.	Jan. 20, 1930	277	6
Feb. 20, 1930	21				
Mar. 13, 1930		First.	[b]		

[a] Abortion.

[b] Ovum recovered.

The heat period of February 20, 1930, was allowed to pass without breeding the cow in order that the post-partum involution of the organs might be completed and a new cycle established. The animal was

bred at 10.05 and 10.10 a. m., March 13, 1930, to two different males. After the first mating, a few sperms, all active, were recovered; after the second, many sperms were recovered, of which 70 per cent were active. Early in the morning of March 15, 1930, the oestral bleeding was profuse. Within a few hours the animal was killed, and contrary to the prevailing opinion that profuse bleeding means sterility in a cow, a fertilized egg was found in the Fallopian tube.

The genital organs were removed aseptically and placed in a sterile, well-insulated, prewarmed box and taken to the Carnegie laboratory. A 2-celled ovum was recovered at 1.10 p. m. from the Fallopian tube. The uterus was found to be filled with a golden-yellow viscous fluid thicker than the white of a hen's egg. Its pH was 9 to 9.2, or 2 points more alkaline than blood, i. e., about that of egg white. The egg was placed on a slide in sterile cow plasma and photographed, at various magnifications, by means of a microscope and a motion-picture camera designed for such purposes. As no further division of the blastomeres took place, the egg was fixed in Bouin's fluid at 5.30 p. m. for further microscopic study. The egg is described later (p. 634).

Cow A–15 was born October 27, 1923. Her record as a breeder is not so good as that of cow A–11, for 12 matings produced only 4 pregnancies. The first pregnancy terminated in an abortion, and the cycles thereafter were more or less irregular, the heats appearing at intervals of 41, 27, 20, 27, 26, 20, and 22 days. The cow was mated six times before she conceived again. Conception followed at the end of the 22-day cycle. Following the termination of the second pregnancy the cycles were 20, 82, 57, 25, and 42 days, conception taking place at the third mating. The next cycles were 21, 24, and 63 days, the second mating resulting in pregnancy. Some of these extended cycles are probably more accurately divisible into shorter ones that passed without the usual signs of heat becoming evident to the attendant. Thus the last two conceptions followed a 42-day and a 63-day cycle, respectively, both numbers being multiples of 21. Perhaps all of the four conceptions may have occurred at the end of typical 21-day cycles. The genital history of Cow A–15 is given in Table 2.

With the expectation of securing a 72-hour ovum, cow A–15 was bred at 8.15 a. m., June 8, 1930, and killed at 9.19 a. m., three days later. There had been no noticeable bleeding of oestrum. On opening the abdomen it was seen that ovulation had not occurred. Evidently the meagerness of the bleeding was associated with the absence of ovulation in this case. The ovaries measured 32 by 27 by 25 millimeters and 40 by 28 by 25 millimeters. One had a large follicle about 20 by 20 by 15 millimeters, and the other had two follicles measuring about 20 by 18 by 16 millimeters each. The walls of all the follicles were thick, yellow, and opaque; the liquor folliculi was yellowish and viscous and there were no ova with discus proligerus. All these phenomena are signs of advanced atresia. No recognizable corpus albicans was seen, which indicated that ovulation had not taken place since the last pregnancy. The uterine lumen contained no surplus fluid.

TABLE 2.—*Genital history of cow No. A–15*

Dates of heats and breedings	Interval between observed heats	Service	Date of calving	Length of gestation period	Pregnancy No.
	Days			*Days*	
Feb. 6, 1925	(?)	First.	Sept. 18, 1925	[a] 234	1
Nov. 3, 1925					
	41				
Dec. 14, 1925		First.			
	27				
Jan. 10, 1926		Second.			
	20				
Jan. 30, 1926		Third.			
	27				
Feb. 26, 1926		Fourth.			
	26				
Mar. 24, 1926					
	20				
Apr. 13, 1926		Fifth.			
	22				
May 5, 1926		Sixth.	Feb. 9, 1927	280	2
May 11, 1927					
	20				
May 31, 1927					
	82				
Aug. 21, 1927		First.			
	57				
Oct. 17, 1927					
	25				
Nov. 11, 1927		Second.			
	42				
Dec. 23, 1927		Third.	Oct. 5, 1928	287	3
Nov. 7, 1928					
	21				
Nov. 28, 1928					
	24				
Dec. 22, 1928		First.			
	63				
Feb. 23, 1929		Second.	Dec. 10, 1929	290	4
Jan. 30, 1930					
	48				
Mar. 19, 1930					
	81				
June 8, 1930		First.	[b]		

[a] Abortion.

[b] Did not ovulate.

Cow No. 411 was born April 17, 1918. The breeding records for her first three pregnancies are not complete. During her fourth sexual season 4 heat periods appeared at intervals of 25, 32, and 24 days; conception taking place with the third mating at the end of the 24-day cycle. Six heats were observed at intervals of 35, 17, 26, 25, and 25 days during the fifth sexual season. Conception occurred at the end of the 25-day cycle with the second service. Following the termination of the fifth pregnancy the cycles were 27 and 26 days, respectively, conception taking place with one mating at the end of the 26-day cycle. The next cycles were 37 and 24 days, respectively, the first mating at the end of the 24-day cycle resulting in pregnancy.

During the eighth sexual season 8 heats were observed at intervals of 16, 24, 24, 22, 25, 22, and 23 days. Five of the six matings during this season were with bulls whose semen was found to have only inactive spermatozoa. Conception occurred at the end of the 23-day cycle when mated with a normal young bull. Two heats, 33 days apart, occurred just before the ninth pregnancy. The first mating at the end of the 33-day cycle resulted in pregnancy. Three heats

appeared at intervals of 27 and 46 days during the tenth sexual season, conception taking place at the end of the 46-day cycle with the first mating. After the termination of the tenth pregnancy, the second heat was selected for the experiment, as in the case of cow No. A–11. The genital history of cow No. 411 is given in Table 3.

TABLE 3.—*Genital history of cow No. 411*

Dates of heats and breedings	Interval between observed heats	Service	Date of calving	Length of gestation period	Pregnancy No.
May 1, 1922	*Days* (?)	(?)	Feb. 6, 1923	*Days* 281	3
Apr. 12, 1923	25				
May 7, 1923	32	First.			
June 8, 1923	24	Second.			
July 2, 1923		Third.	Apr. 6, 1924	278	4
May 14, 1924	35				
June 18, 1924	17				
July 5, 1924	26				
July 31, 1924	25				
Aug. 25, 1924	25	First.			
Sept. 19, 1924		Second.	June 29, 1925	283	5
Aug. 3, 1925	27				
Aug. 30, 1925	26				
Sept. 25, 1925		First.	July 6, 1926	284	6
July 20, 1926	37				
Aug. 26, 1926	24				
Sept. 19, 1926		First.	June 28, 1927	282	7
July 13, 1927	16				
July 29, 1927	24				
Aug. 22, 1927	24	First.			
Sept. 15, 1927	22	Second.			
Oct. 7, 1927	25	Third.			
Nov. 1, 1927	22	Fourth.			
Nov. 23, 1927	23	Fifth.			
Dec. 16, 1927		Sixth.	Sept. 29, 1928	288	8
Oct. 30, 1928	33				
Dec. 2, 1928		First.	Sept. 15, 1929	287	9
Sept. 27, 1929	27				
Oct. 24, 1929	46				
Dec. 9, 1929		First.	Sept. 19, 1930	284	10
Oct. 1, 1930	19				
Oct. 20, 1930		First.	(a)		

[a] Unfertilized ovum.

In order to try again to obtain a 72-hour ovum, this cow was bred to several young bulls, at about 9 a. m. on October 20, 1930, and killed at 9.46 a. m. three days later.

The uterus was removed aseptically and placed in a warm box and transported to the Baltimore laboratory as quickly as possible. At 11.30 a. m., an ovum was recovered by irrigation of the Fallopian tube. Very little salt solution was required to flush out the ovum, which indicated it had traveled far down the isthmus of the tube. At any rate the experiment indicated that at least 74 hours are required for the tubal journey. The ovum had not been fertilized. Evidence is accumulating that the mammalian egg is fertilizable for only a few hours (Lewis (*14*), Hartman (*7*), Parker (*15*)), so perhaps it had been ovulated some time before mating. A description of the egg is given on page 634.

The left ovary contained the corpus luteum with bright-red stigma of large size (8 by 6 millimeters) and bright-red color, about as described in the Kupfer monograph of the ovary of the cow (*13a*). No old corpus luteum was seen; hence, it is likely that ovulation did not take place at the preceding heat period, and mating at that time would, therefore, have been sterile.

The uterus contained no excess of fluid, but only the usual secretion which bathes mucous membranes. The pH of this secretion was 7 to 7.2, or about that of blood, and 2 points more acid than in the case of cow No. A–11. The mucous secretion of the uterus had apparently been emptied into the vagina, which still contained a considerable amount of yellowish viscous fluid streaked with blood. The pH of this fluid was also 7 to 7.2. There was no evidence of the source of the oestral bleeding, which was quite meager in this case.

THE EGG

Two eggs were obtained. The first (No. 1, from cow No. A–11), was fertilized and in the 2-cell stage when recovered. The second (No. 2, from cow No. 411) was unfertilized. As seen from Figure 1, the cow egg is typical of mammalian eggs in general—in size, shape, zona radiata, and character of the cytoplasm. The fat granules are fine and evenly distributed throughout the cytoplasm, i. e., no polarity is visible. Neither polar body was differentiable with the methods employed in the case of egg No. 1; egg No. 2 had but one polar body, as is to be expected in the case of an unfertilized egg. The zona of egg No. 1 in the fresh state measured 15 microns in diameter (fig. 1, B), but in alcohol after fixation it shrank to 4.5 microns (fig. 1, E). The zona of egg No. 2 in the fresh state was a little thinner, or 12 microns. (Fig. 1, A.) Other measurements of egg No. 1, the 48-hour, 2-celled cow egg, are recorded below.

From considerable experience in the Carnegie laboratory it has been found that measurements of such small objects as mammalian eggs are best made from photomicrographs taken at a known magnification that is sufficiently great to reduce the error of measurement to a minimum. Furthermore, as Hartman (*8*) pointed out, reliable data on the size of the egg are to be secured only from the living egg immersed in a suitable medium, for fixation and dehydration greatly shrink the egg, as was strikingly shown in the present case. Hartman indicated that most dimensions given for mammalian eggs in the past

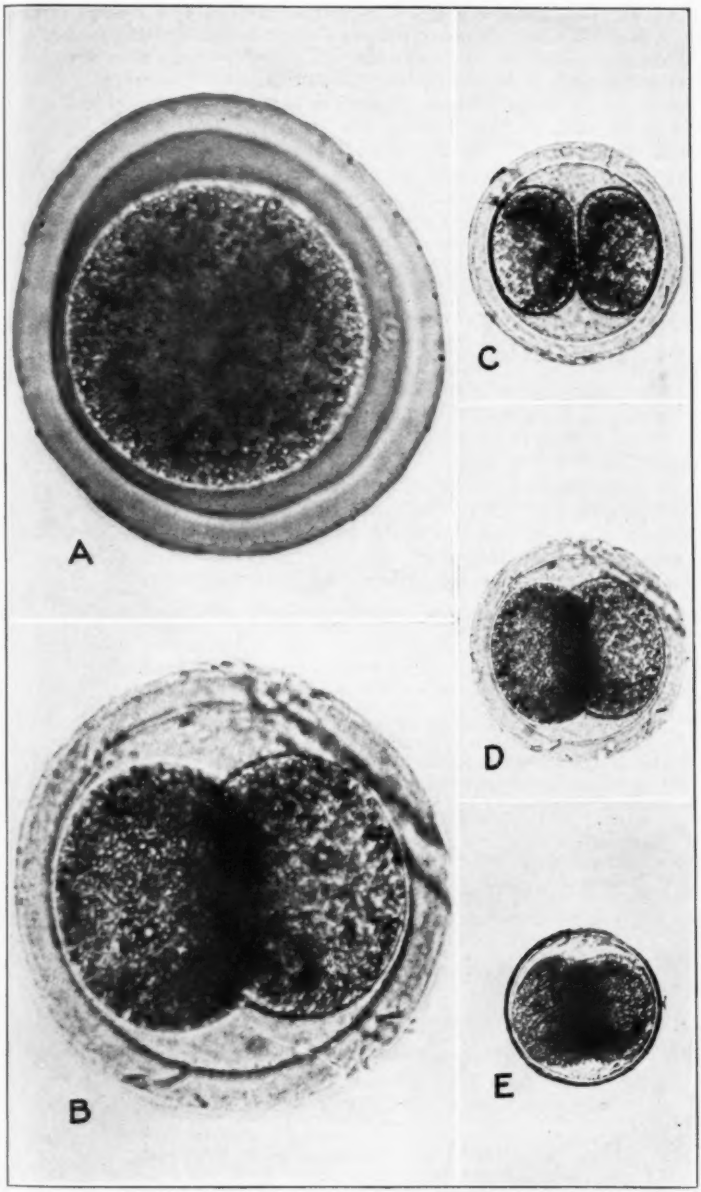

FIGURE 1.—Photomicrographs of the cow egg: A, Unfertilized egg No. 2, obtained 74 hours post coitum from cow No. 411; B to E, fertilized, 2-cell egg No. 1, obtained 51 hours post coitum from cow No. A–11. A to D, photographed alive in cow plasma; E, photographed after fixation in alcohol to show shrinkage. A and B magnified 400 diameters; C, D, and E magnified 200 diameters

71917—31——5

were too low. He estimated the diameter of the living horse egg at 135, that of the sheep at 120, of the goat at 140, and the egg of the pig at from 120 to 140 microns. The cow's egg, according to the present study, is about 135 to 140 microns in diameter. The size of the egg, it should be explained, is taken as the inner diameter of the zona; for in the freshly discharged ovum, the cytoplasm or vitellus completely fills the zona. The zona is, as a matter of fact, laid down around the egg in the ovary, probably by the egg itself. Soon after the discharge of the egg, and especially after fertilization, the vitellus shrinks, probably because of the elimination of water, leaving a clear perivitelline space of varying size about the cells or blastomeres.

SIZE OF EGG NO. 1, FRESH. (FIG. 1, B)

Dimensions inside zona: 135 by 135.5 microns.
Volume: 1,600,000 cubic microns.
Dimensions outside zona: 164.8 by 165 microns.

SIZE OF EGG NO. 1, FIXED. (FIG. 1, E)

Diameter inside zona: 110 microns.
Volume: 698,775 cubic microns.
Volume shrinkage 56 per cent as compared with the fresh egg.

If, now, we wish to ascertain the dimensions of the cells from the photographs (fig. 1, C and D) we encounter some difficulty, for C is a direct side view and shows the two more or less biscuit-shaped cells closely appressed together, whereas in D the cells overlap. The following calculations are approximately correct:

SIZE OF THE LIVING CELLS FROM EGG NO. 1. (FIG. 1, C)

One cell:
Dimensions: 97 by 64.9 microns; average 86 microns.
Volume: 333,000 cubic microns.
The other cell:
Dimensions: 99 by 65 microns; average 87 microns.
Volume: 346,000 cubic microns.
Total volume: 679,000 cubic microns.
Volume shrinkage in first cleavage: 58 per cent.

SIZE OF LIVING CELLS FROM EGG NO. 1. (FIG. 1, D)

Each cell:
Dimensions: 103 by 67.5 (one-half of 135) microns; average 91 microns.
Volume of each: 400,000 cubic microns.
Total volume: 800,000 cubic microns.
Volume shrinkage in first cleavage according to this measurement, 50 per cent.

SIZE OF FIXED CELLS FROM EGG NO. 1. (FIG. 1, E)

Each cell:
Dimensions: 80 by 55 (one-half of 110) microns; average 72 microns.
Volume: 197,000 cubic microns.
Volume shrinkage as compared with the living cells: Approximately 70 per cent.

SIZE OF 74-HOUR NON-FERTILE COW EGG NO. 2. (FIG. 1, A)

Inside zona:
Dimensions: 140 by 145 microns; average 143 microns.
Volume: 1,537,000 cubic microns.
Vitellus:
Diameter: 120 microns.
Volume: 907,200 cubic microns.
Volume shrinkage of vitellus: 41 per cent.

If one wishes to visualize how small a cow's egg really is (Hartman (*9*)) he should scatter a pinch of sea sand on a sheet of black paper. The smallest grain visible to the naked eye is about the size of the cow's egg. It would require about 2,000,000 cow eggs to fill a thimble of about 3 cubic centimeters capacity.

SUMMARY AND CONCLUSION

The recovery from cow No. A–11 of a fertilized egg in the 2-cell stage 48 hours after a breeding during oestrum indicates that both ovulation and fertilization may be completed within 48 hours after the appearance of oestrum in the cow.

Evidence that the second cow (No. A–15) had not ovulated shows that oestrum may not necessarily be accompanied by ovulation. This may explain why some matings during oestrum do not result in conception.

The recovery of an unfertilized ovum in the Fallopian tube of cow No. 411 shows definitely that she ovulated at this oestrum, but that, although she was mated to several young bulls known to have active spermatozoa, fertilization did not take place. Whether failure to conceive in this case was due to mating at the incorrect time, or to some physiological condition unfavorable to fertilization, is not known. Failure to get conception by mating cows in heat to bulls producing active spermatozoa is illustrated.

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SOME FACTORS CONCERNING EARLINESS IN COTTON[1]

By C. A. LUDWIG,
Formerly *Associate Botanist and Plant Pathologist, South Carolina Agricultural Experiment Station*

INTRODUCTION

Since the advent of the Mexican boll weevil in the United States, cotton production in the infested sections has become an annual race with this insect. If the weevil multiplies enough before a fair crop of cotton is set and past the easily susceptible stage, the crop is a failure. If not, a fair or good crop can be made. The weevil may be largely circumvented either by holding it in check or by producing an early crop, or both. Under such conditions anything conducive to earliness is important. Because of this importance a few of the factors which seemed likely to have a bearing on earliness were selected for this study, and their influence was investigated.

METHODS

The data on which the following discussion is based were collected in the summers of 1923 and 1924 at the South Carolina experiment station farm at Clemson College, and the calculations and a tentative draft of the text were made by 1927. However, the manuscript was not completed at that time because work on it was interrupted by the removal of the writer to another location and to another line of investigation.

The following terminology will be used in this paper. A square is a blossom bud, a boll is the structure which follows a bloom, and a form is either a square or a boll. Squaring is the initiation of floral buds, or "squares." The square period is the time elapsing between the first observation of a square and its opening as a flower. The boll period is the time from flower opening to the opening of the boll. A square is usually a millimeter or so in diameter, and the subtending leaf is about ready to unfold by the time it is detectable. The square period as here defined is therefore not the true square period, which would be the time from the laying down of the primordium of the square until its opening as a flower. Bailey and Trought (*3, p. 3–8*),[2] by dissecting young sympodia and by extrapolating curves of the growth of squares, have determined that young squares become detectable on Egyptian cotton in Egypt at about 9 days of age. So far as the writer is aware no one has yet done this for any variety of upland cotton. For the purpose of this paper such a determination is unnecessary, as the comparisons can be made just as well without it.

The plan followed was to keep a record of each form from the time it became large enough to be recognized until it opened as a boll or was shed. The observations were made every second day except at week-ends, when two days intervened, and when rain interfered,

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[2] Reference is made by number (italic) to Literature Cited, p. 657.

which was not very often. Ten plants were tagged as a sample in each case, care being taken to get plants which were as nearly alike as possible in size, vigor, and environment except for the feature to be varied. Each leaf axil was numbered, beginning at the ground; and each square, when observed, was indicated on its appropriate branch on a chart of the plant. Later the dates of blooming and opening of boll or of shedding were recorded. During 1924 each form was tagged at blooming time with the date of bloom. It was thus possible to be very sure which bolls remained on the plants to maturity and which were shed. At the end of the season the records were transferred to cards, the subtractions made to determine square periods, boll periods, etc., and the means and probable errors of these values were computed for each treatment in the tests. It should be remembered that any method of making observations at any except daily intervals introduces an error into the results. It can be shown that for observations taken every second day the effect is to make the square period one-half day too short and the boll period one-half day too long. With observations taken at longer intervals the error is greater. However, no account has been taken of these errors in this report, as they do not affect the comparisons made. It may perhaps not be out of place to mention that apparently most square and boll period determinations previously published are affected by such errors.

The variety used throughout, unless otherwise stated, was Wannamaker's Cleveland in 1923, and the same or another strain of Cleveland in 1924. The plants were grown at Clemson College, South Carolina, on a Cecil sandy loam type of soil and the treatments applied (except for the stripping of squares) by the agronomy division of the experiment station, which was conducting somewhat similar studies. The boll weevil was kept practically absent until well after migration by frequent applications of calcium arsenate dust. The usual cultural practices were followed except where altered for experimental purposes or to overcome the effect of the excessive tramping occasioned by taking frequent records. The plants made good growth except during the dry weather in 1923 and except for a certain amount of stunting apparently due to excessive handling. The latter part of the season of 1923 was very dry, and much shedding occurred, apparently as a result of this condition. More rain fell in 1924, however, and it was better distributed.

It will frequently be noted in the discussion following that in many instances the analysis of the 1923 data is carried out in considerably more detail than that of the 1924 data. This is because the original 1924 data were destroyed in a fire in the agricultural hall at Clemson College on April 2, 1925. Those calculations that had not already been completed could be made only on the 1923 data, which, fortunately, were saved.

RESULTS

The following topics have been investigated and are considered here: (1) Varietal differences in the maturation periods of squares and bolls; (2) effect on the maturation periods of the time of application of nitrogenous fertilizer; (3) effect of the amount of nitrogenous fertilizer applied; (4) effect of the spacing of the plants; (5) effect of duration of cultivation; (6) effect of stripping the forms; and (7) the rate of development of the bolls.

VARIETAL VARIATIONS IN SQUARE AND BOLL PERIODS

The five varieties, Cleveland, Trice, King, Webber 49–6, and Lightning Express were selected for Study. The seed was planted about May 28 in 1923, and on May 7 in 1924, in single rows with a number of other varieties not included in this study. Table 1 gives the results secured for the varieties concerned for the two years.

TABLE 1.—*Blooms, square periods, bolls, and boll periods for five varieties of cotton in 1923 and 1924*

Variety	Year	Blooms	Square period	Bolls	Boll period	Boll period (Blackwell and Buie)
		Number	*Days*	*Number*	*Days*	*Days*
Trice	1923	304	22.00±0.12	112	54.39±0.34	54.17
	1924	154	25.15± .18	84	65.21± .72	
King	1923	270	22.80± .15	120	56.08± .39	55.77
	1924	161	26.02± .15	80	60.59± .54	
Webber 49–6	1923	270	22.33± .13	96	58.37± .52	57.04
	1924	222	25.39± .14	116	65.69± .63	
Lightning Express	1923	204	22.73± .16	122	54.84± .44	56.83
	1924	370	24.98± .10	233	65.51± .49	
Cleveland	1923	242	22.29± .14	113	52.19± .25	54.08
	1924	221	26.00± .16	116	63.32± .55	
Cleveland [1]	1923		22.27± .04		54.66± .10	54.08
	1924	3,242	26.27± .03	1,509	61.57± .15	

[1] Average for all Cleveland plants, 160 in number, except those that were stripped, grown in all the tests reported in this paper.

This table shows that the range of square periods for all varieties is from 22.00 ± 0.12 to 22.80 ± 0.15 days for 1923, and from 24.98 ± 0.10 to 26.27 ± 0.03 days for 1924. Thus the differences for any one season are not great. The difference between the two extreme values in 1923, omitting the combined Cleveland results, which are not comparable here, was only 0.80 ± 0.19 day and in 1924 only 104 ± 0.18 days. While according to the calculated probable errors these differences and some of the others are significant, other considerations, discussed in connection with the boll periods, give reason for caution in emphasizing the differences found. It is perhaps worth noting, though, that Trice ranked first or second both years in shortness of square periods while King ranked last. The ranking of the other varieties varied. However, the results indicate that there is little variation among the varieties used.

It will be of value to compare these square periods with those reported by other investigators. What appears to be the first accurately determined square periods were reported in 1896 by Hammond (*20, p. 265*) as having been determined in 1887 in South Carolina. The variety was not stated. The values fluctuated between 24 and 25 days for different months of the season. Martin, Ballard, and Simpson (*30, p. 200*) have given 28.45, 23.20, 22.80, and 23.40 days, respectively, for Meade in South Carolina, and Lone Star, Acala, and Durango in Arizona. McNamara, Hubbard, and Beckett (*29, p. 9*) have reported a square period of 27.12 days (mean for thinned and unthinned cotton for four different planting dates) for Lone Star in Texas; while Buie (*16, p. 47*) found 23.6 to 25.0, 26.1 to 28.1, and 26.8 to 28.0 days for six varieties of upland cotton in South Carolina in 1925, 1926, and 1927, respectively. These values for upland cotton

are slightly higher on the average than those reported in this study but not more so than could easily be due to normal variation from season to season.

Balls (*6, p. 59*) gave 23 days as the period of square development for "No. 77 strain" at Giza, but it is not clear how the value was obtained. He also (*7, p. 174–183*) gave 29 days as the period for Assili in 1912 and 27 days for Domains Afifi in 1913. These results are probably not far from correct but must be considered as only rough approximations, as they were obtained by matching the irregularities of the blooming curves with those of the displaced vegetative-growth curve. Experience indicates that the method is unreliable because the variations among the periods of individual squares are too great to permit day-to-day variations in plant growth to be projected with any accuracy into the blooming frequencies some weeks later. Prescott (*32, p. 36*) marked buds of Egyptian cotton, apparently of the variety Sakellaridis, on three different dates in 1922 and under five different conditions as to irrigation, and determined the square periods for the different lots. The mean of his values is 25.8 days. Bailey and Trought (*3, p. 3–8*) found values ranging from 32.3 to 33.6 days for the varieties Assili and Sakel in Egypt. Martin, Ballard, and Simpson (*30*), working with the American-Egyptian variety Pima, in the southwest part of the United States found the period to be 30.11 days, while Loomis (*28, p. 9*) found 33.17 days for the same variety in the same section.

What appears to be the only determination of the square period of sea-island cotton has also been made by Martin, Ballard, and Simpson (*30, p. 200*). The figure was 33.04 days for the season of 1922 in South Carolina.

It appears from the foregoing that the square period for upland cottons is significantly and consistently lower than that for Egyptian cottons, which in turn is possibly lower than that for sea island. From the standpoint of rapidity of development of squares, therefore, the upland type of cotton is superior, although only slightly so, to the Egyptian type. It is to be hoped that the square period of other types of cotton will be investigated in the near future under circumstances which will allow more exact comparisons between types and strains. A type with an especially short period, even if undesirable from some other standpoint, might be of distinct value for hybridizing as a first step in developing a new variety.

The variations found among the boll periods are greater. Reference to Table 1 shows that the maximum difference was 6.18 ± 0.58 days in 1923, and 5.1 ± 0.83 days in 1924. The order of the varieties for the two years was almost the same, with Cleveland heading the list and Webber last.

There is little question that these extreme differences reflect inherent characteristics of the varieties concerned, but there is good reason for suspecting that such is not the case for many of the smaller differences, even where the probable errors indicate high odds in their favor. Thus with the Cleveland bolls in 1923 there was a difference of 2.47 ± 0.27 days between the 10 plants in the variety test plot and the total of 160 plants on which data were secured. The corresponding difference in 1924 was 1.85 ± 0.57 days. The size of the probable errors would make these differences appear real. Possibly they are, the differences being due to differences in the environ-

mental conditions of the two lots of plants; but the failure to discover any considerable or clear-cut effect of any of the environmental factors investigated makes it difficult to accept such an interpretation. Further reason for doubting the sufficiency of the probable errors is furnished by some results secured by Blackwell and Buie (*13, p. 41, 43*) on plants of the same varieties and grown in the same test rows as these reported here. These authors were not considering square development at that time and consequently were able to use a simpler system of collecting data. They were thus enabled to use larger numbers of plants, 50 per group, and to make daily records (except perhaps at week-ends). Their results, shown in the last column of Table 1, should therefore be the more accurate. The probable errors were not reported, but it seems safe to consider them at the most no greater than the ones found in the present study, where the boll numbers were much smaller. If the two sets of results are compared on the basis of the calculated and assumed probable errors, it appears that for some of the varieties they do not differ significantly. The opposite is the case, however, for Lightning Express and Cleveland, especially if the present results be corrected for the error due to the method of making observations. But when it is remembered that in each case the plants under comparison, although not identical, grew in the same rather short row it is evident that observed differences can hardly be real in the sense of having their source in the characteristics of the population sampled. In this case the calculated probable errors as usually used are therefore clearly insufficient as criteria of innate differences among the groups of plants sampled.

It is probably true also that the bolls from lots of 10 plants each do not constitute large enough samples for detecting small differences between groups. In later work the forms from 40-plant lots have been used as samples.

An additional possible source of error lies in the possibility that the bulk of the crop is produced enough later in some varieties than in others to make a difference in the calculated boll period, since late bolls take longer to mature than early ones.

The range of mean boll periods according to Blackwell and Buie is from 54.08 to 57.04 days; while the one here reported is greater, from 52.19 to 58.37. This is, of course, the result that would be expected from the fact that they used the larger groups. The order for the five varieties is the same in both cases except that King and Lightning Express exchange places. For 16 other varieties Blackwell and Buie reported periods ranging from 53.24 to 69.18 days. Buie (*16, p. 22, 45–49*) has since supplemented this report with values for 1925 for approximately the same list of varieties, and for the years 1925, 1926, and 1927 for six of them. The range in values for the entire group for 1925 is from 42.64 to 47.51 days. For the smaller list the ranges were 42.7 to 48.2 days for 1925, 57.7 to 71.3 for 1926, and 54.9 to 62.1 for 1927.

Ewing in 1918 (*19, p. 86–89*) published some valuable data on varietal variation in boll periods in Mississippi. He did not include an entire season's bolls but only those from blooms which opened during a period of about two or three weeks in July. However, a good many varieties were studied for three years, 1911, 1912, and

1914; and the evidence as regards varietal variation agrees definitely with the South Carolina data.

These two sets of results constitute perhaps the largest body of data on this point yet assembled for a series of cotton varieties grown in the same locality in different years. They demonstrate a number of things which may perhaps be more conveniently summarized here than later on. In the first place, they show considerable variation among the varieties each season. In the second place, a certain amount of fixity of type among the varieties appears from season to season. The order of varieties on the basis of the maturation period from season to season is not identical, but on the other hand is not greatly different. The variety that is first one time is not last another time, nor is the last first. As stated by Buie (*16, p. 41*), "there is a rather definite boll period for each of the several varieties which is not entirely obscured by seasonal variations." This is particularly noticeable in the Mississippi results. By way of corollary it would seem to follow that it would be practical by the usual plant-breeding technic to produce strains with notably short boll-maturation periods. Care would need to be exercised, of course, not to sacrifice other desirable characteristics. Ewing points out, for instance, that shortness of boll-maturation period is associated in general with small bolls; but he is careful to state that this is not universally the case. In the third place, the results show that season-to-season variations in the boll period are often more striking than varietal variations. However, this last point will not be discussed here.

A number of other determinations of the boll period have been made. What appears to be the earliest of these was made in 1887 in South Carolina (*20, p. 265*). The periods ranged from 45 to 71 days for different parts of the season, but the mean for the season was not given. Bryan and Pressley (*14, p. 5, 8*) determined the boll periods for the varieties Acala, Hartsville, Mebane, and Deltatype Webber for 1923 in Arizona. These values varied from 54 days for Acala to 63 days for Hartsville.

King, Loomis, and Varmette (*25, p. 952*) report 62.3 days for Pima and 47.1 to 54.3 days for five varieties of upland cotton in Arizona. Loomis (*28, p. 26*) gives "about 58 days on Pima and about 53 days on Acala in 1924 and about 62 days on Pima and 57 days on Acala in 1925." So far as the writer is aware, the only other determinations of the boll period of an American-Egyptian cotton have been reported by King (*24, p. 5–6*). The value determined for 3,319 bolls of Pima cotton at Phoenix, Ariz., in 1919 was 68 days. He further reports an otherwise unpublished finding of 67 days by Leding for 1918 at Sacaton, Ariz.

Beckett (*11, p. 104–106*) reported 45.49 days as the boll period of the variety Garo Hill, of *Gossypium cernuum*, an Asiatic cotton, and 40.83 days as that of Lone Star at Greenville, Tex., in 1924. While the boll period of this Asiatic cotton is thus about the same as that of our upland varieties, the author calls attention to the fact that it is one of the larger bolled varieties of this class and that smaller bolled varieties might show a shorter boll-maturation period. This suggestion gives added interest to some results secured by Allard (*1, p. 127–130*), who reported on the periods of several varieties for a limited portion of the season in north Georgia. The varieties included some foreign cottons, one of which had the shortest period of those tested.

The periods given are as follows: Moki, 48.1; Okra-leaved, 54.9; Willet's Red, 54.9; Murasaki (Japanese), 44.9; and Hawasaki (Japanese), 47.3 days.

Balls was one of the first to record the length of the boll maturation period for any Egyptian variety of cotton. For "King Upland," Egyptian Sultani, and Mit Afifi at Cairo (*5, p. 66*) he gave 42, 51, and 48 days, and for Domains Afifi and "Strain No. 77" at Giza in 1913 (*7, p. 198–200*), 51 and 48 days, respectively. He was apparently of the opinion that the period does not vary from year to year. It is possible that for Egyptian conditions this opinion is substantially true, but for humid regions it can hardly be correct. Prescott (*32, p. 36*) determined the maturation period of bolls of the variety Sakellaridis from flowers of three different dates from June 21 to July 22 and under five different conditions of irrigation. The unweighted mean of the values reported is 51.5 days. Bailey and Trought (*3, p. 8–14*) found 52 days as the period for healthy Sakel (strain 4/20) bolls in Egypt from flowers opening between June 10 and August 10, 1921. A limited number of Assili bolls in 1922 gave 47.9 days as the mean. Lambert (*27, p. 59*) reported a maturation period of about 60 days for bolls of Massey's Domains Sakel (Shambat) in the Sudan during the season 1927–28. This was said to be a shorter period than usual, but the same figure had been given for 1926–27 (*26, p. 46*).

Harland (*21, p. 191–193*) found the maturation period for 144 healthy sea-island bolls in St. Vincent to be 51.2 days. Two plants of Southern Cross, an upland variety, in rich ground gave maturations periods of 49.5 and 48.5 days; two plants in poor ground gave 45.3 and 46.3 days. Elsewhere (*23*) he reported "30 days in some Asiatic cotton, to 51 in 'sea island'" in the West Indies, where the temperature conditions are at or near the optimum. In a greenhouse in England, sea island was observed to take up to 70 days. Mason (*31, p. 188*) reported 51 days as the mean maturation period for sea-island bolls in St. Vincent, but he was apparently merely repeating Harland's figure. Martin, Ballard, and Simpson (*30, p. 206*) found a period of 56.1 days for Meade and 57.6 days for sea-island bolls in South Carolina.

According to the forementioned results the boll maturation periods of upland varieties of cotton appear to be shorter, in general, than those of Egyptian or sea island. The range seems to overlap, however; the more slowly maturing upland varieties are probably no better in this respect than the others. The Asiatic cottons which have been reported thus far appear to rank with the upland cottons in this regard, and it is possible that some other varieties of this group will be found even more rapid in maturing.

EFFECT ON SQUARE AND BOLL PERIODS OF VARIATIONS IN THE DATE OF APPLICATION OF NITRATE

The seed for this test was planted about May 21 in 1923, and on May 9 in 1924. The field used in the former year sloped to the north, and the one used the next year sloped to the south. All plots were given a 500-pound dressing of a 4–10–2[3] fertilizer applied at planting. The experimental application of nitrate of soda was made at the rate of 100 pounds per acre. Table 2 gives the results secured from the two seasons' work.

[3] The probable formula. It can not be given with certainty, owing to the destruction of records in the fire. The figures refer to NH_3, P_2O_5, and K_2O, respectively.

TABLE 2.—*The effect of the application of nitrate of soda to cotton plants at different dates on the duration of the square and the boll periods, 1923 and 1924*

Date of applying nitrate	Blooms	Square period	Bolls	Boll period
1923	*Number*	*Days*	*Number*	*Days*
June 20	194	22.60±0.18	90	55.99±0.43
July 1	180	22.18± .15	84	54.51± .44
July 16	160	21.81± .18	70	55.01± .45
1924				
At planting	171	26.05± .13	70	56.29± .51
June 12	148	26.45± .11	7[1]	55.97± .53
June 26	168	25.62± .11	68	59.32± .69
July 10	216	25.86± .12	100	58.21± .53

The data given indicate that any effect on the length of the square and boll periods due to varying the time of application of nitrogen to cotton is relatively insignificant. The square periods for each year taken alone show a general tendency downward as the application of nitrogen is delayed. When the results for the two years are averaged, matching June 20, 1923, with June 12, 1924, etc., the values secured are 24.5, 23.94, and 23.84 days, respectively. The extreme difference is 0.7 day. This value, while possibly significant, since the general trend agrees for the two years, is very doubtfully so and certainly is not sufficient to be of any importance in planning fertilization methods. It is of some theroetical importance if really significant, as suggesting that plenty of available nitrogen while the plant is young tends to make the squares develop more slowly. It should be noted in this connection that even the late applications were made before the large majority of flowers had opened and probably before the primordia of some of the later ones had been laid down.

In the matter of the boll period the evidence does not indicate any influence due to the time of application of the nitrate. It does not show a clear-cut tendency for either year; and the evidence of one year, such as it is, contradicts that of the other. When we consider that the last application occurred before many bolls had reached any considerable size, it is clear that practically all the bolls in every plot developed after the application of nitrate to the plants on which they were produced. The results, therefore, do not show what would happen in case of such a delay in applying nitrogen as would permit a good many bolls to develop before the application.

EFFECT ON SQUARE AND BOLL PERIODS OF DIFFERENT RATES OF APPLYING NITRATE

The seed for this test was planted about May 28 in 1923, and on May 7 in 1924. All plots had a basic application of 500 pounds of a 4–10–2 [4] fertilizer per acre. The test applications of nitrogen were in addition to this. They were given in the form of nitrate of soda and were applied about July 1. The results obtained as to square and boll periods are given in Table 3.

An examination of Table 3 reveals no connection between the amount of nitrogen applied to cotton and the length of the square period. The results for each year are erratic, as well as the averages for both years. The 2-year averages, beginning with the checks, are as follows: 24.58, 24.80, 24.12, and 24.44 days.

[4] See footnote 3.

TABLE 3.—*The effect of different rates of application of nitrate of soda to cotton plants on the duration of the square and the boll periods, 1923 and 1924*

Year and quantity of nitrate applied	Blooms	Square period	Bolls	Boll period
1923	*Number*	*Days*	*Number*	*Days*
Check	220	22.64±0.16	90	54.56±0.45
100 pounds	241	23.10± .15	96	57.12± .51
200 pounds	218	22.73± .16	110	54.80± .38
300 pounds	211	23.00± .14	97	53.83± .36
1924				
Check	188	26.51± .13	92	62.68± .60
100 pounds	189	26.50± .12	93	59.95± .53
200 pounds	224	25.50± .13	105	61.81± .46
300 pounds	200	25.87± .13	94	58.60± .44

The boll-period results are also erratic, although showing some evidence of a decrease as the application of nitrate is increased. The 2-year averages in this case are 58.62, 58.54, 58.31, and 56.22 days, respectively. This result is in harmony with the finding reported by Blackwell and Buie (*13, p. 49*) that earliness is correlated with nitrogen applications to the soil. It suggests that shortening of the boll period may partially account for the earliness which they observed. The amount of shortening is too small to account for any great increase in earliness, however, especially in the light of the results secured by Ewing (*19, p. 41*), who investigated the boll periods of cotton on a fertile soil and on a poor soil near by during the summers of 1912 and 1914. In 1912 he found that the period on the fertile soil was 3.45 days longer than on the unproductive soil. In 1914 the same qualitative difference was noted, but it was so small as not to be considered significant. The results obtained from a few plants by Harland (*21, p. 191–193*) agree with those of Ewing. These results, it will be noted, are the opposite of those reported here. The stimulation of growth, resulting in early fruiting or at least the retention of more early bolls, is probably of much greater importance than any possible shortening of the boll period in producing an early crop, particularly as Buie (*15, p. 200*) and Buie and Warner (*17, p. 27–28*) have reported that nitrogen stimulates early fruiting on light soils. It is, of course, a matter of common observation that a greater percentage of the final crop is laid down early on fertile soils than on poor ones.

A repetition of this experiment, with a much greater range of nitrogen applications, is needed.

EFFECT ON SQUARE AND BOLL PERIODS OF THE SPACING OF THE PLANTS

The seed for this test was planted about May 28 in 1923, and on May 7 in 1924. A 4–10–2 [5] fertilizer was applied to all plots at the rate of 500 pounds per acre at planting and a side application of 100 pounds of nitrate of soda about July 1. The spaces between plants which were investigated were 3½ feet between rows, unthinned, 3½ feet by approximately 8 inches, 3½ feet by approximately 16 inches, and 3½ feet by approximately 24 inches. The results are given in Table 4.

[5] See footnote 3.

TABLE 4.—*The effect of different spacings of cotton plants on the number of blooms and bolls produced, and on the duration of the square and the boll periods, 1923 and 1924*

Year and spacing	Blooms	Square period	Bolls	Boll period
1923	*Number*	*Days*	*Number*	*Days*
3½ feet, unthinned	60	22.45±0.18	35	54.63±0.64
3½ feet by 8 inches	134	22.31± .29	67	55.99± .46
3½ feet by 16 inches	223	21.38± .12	128	54.52± .31
3½ feet by 24 inches	347	21.99± .12	194	55.56± .27
1924				
3½ feet, unthinned	89	26.58± .18	47	62.81± .81
3½ feet by 8 inches	123	26.46± .16	68	60.43± .66
3½ feet by 16 inches	293	26.18± .09	142	56.71± .40
3½ feet by 24 inches	281	26.83± .11	138	62.28± .44

The evidence is clear from these results that variations in the thickness of the stand exerted no appreciable influence on either the square period or the boll period for the season as a whole, in spite of the fact that such variations exert a very appreciable influence on the earliness of the crop. In fact, it is possible, in view of the findings of McNamara, Hubbard, and Beckett (*29, p. 12–14*), that the thinning actually shortened the square and boll periods for the individual forms, but that this effect was small in the case of the squares and masked in the case of the bolls by the greater number of late-season forms matured by the thinned plants, as in the United States a late boll requires more time than an early one to mature. These writers determined the periods for bolls from blooms of three definite dates on thinned and on unthinned plants in Texas in 1924. The mean of the values they give for the thinned plants is 44.13 days and for the unthinned plants, 45.05 days, or a period approximately a day longer for unthinned plants than for thinned ones. It is perhaps worth mentioning that they also determined the square period for the thinned and for the unthinned plants. The means of their figures are 26.69 days for the thinned, and 27.55 days for the unthinned.

Thick spacing is recognized as a practical means of producing an early crop, but it should be kept clearly in mind that it does not shorten the period of maturity of the forms nor cause the individual plants to begin fruiting earlier. It merely furnishes a larger number of plants per acre to produce the early forms and at the same time suppresses most of the vegetative branches, which are the ones usually responsible for much of the later crop. Table 5 brings out the facts in the case very clearly.

The data given in Table 5 are from groups of 10 plants each instead of groups grown on equal areas of land. The wide-spaced plants begin to produce squares and blooms fully as soon as the close-spaced ones do, if not sooner, and very soon they outstrip the latter. It is true that the average date of squaring and blooming is later with the wide-spaced plants; but this result is due to the fact that the production of forms increases more rapidly and is continued longer with them than with the close-spaced plants. It is not due to any delay in initiating the formation of squares.

TABLE 5.—*Squares and blooms observed each week, totals for the season, and earliest, last, and mean dates for squaring and blooming for different spaced cotton plants in 1923*

Date	Squares for spacing indicated				Blooms for spacing indicated			
	3½ feet, unthinned	3½ feet by 8 inches	3½ feet by 16 inches	3½ feet by 24 inches	3½ feet, unthinned	3½ feet by 8 inches	3½ feet by 16 inches	3½ feet by 24 inches
July 6–12	34	43	45	60				
July 13–19	40	50	39	93				
July 20–26	28	51	93	88	1	3	2	9
July 27–Aug. 2	11	39	70	164	8	12	18	18
Aug. 3–9	8	16	67	74	19	35	40	62
Aug. 10–16	5	2	20	71	24	48	90	96
Aug. 17–23		1	8	8	5	27	65	95
Aug. 24–30			2	6		9	19	45
Aug. 31–Sept. 6			1	4			6	17
Sept. 7–14 (8 days)			1	3			5	4
Total	126	202	346	571	57	134	245	346
Earliest	July 6	July 6	July 6	July 7	July 26	July 24	July 23	July 23.
Last	Aug. 16	Aug. 18	Sept. 7	Sept. 14	Aug. 22	Aug. 28	Sept. 14	Sept. 8.
Mean	July 20	July 23	July 28	July 29	Aug. 9	Aug. 12	Aug. 15	Aug. 16.

The facts in the case suggest that squaring begins whenever the plants are large enough, and that close-spaced and wide-spaced plants begin to square at about the same time. After this the development of the plants depends at least to some degree on the space available, but the earliest and largest acre yield is produced at a spacing that does not permit the development of large individual plants.

EFFECT OF DURATION OF CULTIVATION ON SQUARE AND BOLL PERIODS

The seed for this test was planted about May 28 in 1923, and on May 7 in 1924. In the latter year a poor stand was secured, and a replanting was made on June 5. The plot was fertilized with 500 pounds per acre of a 4–10–2 [6] fertilizer at planting and 100 pounds of sodium nitrate as a side application about July 1.

One plot in each season was given no cultivation except the minimum amount of scraping with a hoe necessary to destroy the weeds, the second was given the usual cultivation recognized as good practice, and the third was given a cultivation prolonged considerably later than usual into the season. Thus in 1924 the weeds were scraped off the first plot on June 16 and July 25; the second plot was cultivated on June 16, July 15, and July 25; and the third one was cultivated on these dates and on August 14 and 23 in addition. The results are shown in Table 6.

TABLE 6.—*The effect of different durations of the cultivation period of cotton plants on the duration of the square and the boll periods, 1923 and 1924*

Year and cultivation practice	Blooms	Square period	Bolls	Boll period
1923	*Number*	*Days*	*Number*	*Days*
None	133	21.23±0.17	58	52.80±0.38
As usual	161	22.27± .19	74	53.76± .38
Prolonged	229	22.29± .14	112	56.90± .31
1924				
None	151	25.97± .14	61	68.49± .52
As usual	187	27.04± .12	63	72.24± .73
Prolonged	205	27.07± .11	74	69.90± .68

[6] See footnote 3.

The two seasons' results agree fairly well with each other and indicate an increase in the square period of about one day and in the boll period of about three days due to cultivation. The ordinary cultivation seems to produce more difference as compared with no cultivation than late cultivation does as compared with the ordinary amount. It is possible that an increase in the number of late bolls accounted for most or all of the difference in boll periods, since late bolls have a longer maturation period. It seems possible, also, that both phenomena are associated with differences in soil moisture.

It would seem that a minimum of cultivation might have some value in producing an earlier crop. It would also reduce expenses. The production figures given in Table 6, however, indicate that if carried to extremes it would also decrease yields.

EFFECT OF STRIPPING ON SQUARE AND BOLL PERIODS

The development a few years ago at the Florida experiment station of a method of boll-weevil control (*33*) that involved stripping off all the early forms shortly after the emergence of the last of the hibernating boll weevils [7] made it seem advisable to investigate the reaction of the cotton plant to such treatment in South Carolina. Accordingly 50 plants of Cleveland and 50 of Webber 49–6 were selected in 1923 and again in 1924 and were treated as follows: 10 were given no treatment and served as a check; 10 were stripped of all forms early in the season; 10 were stripped 9 days later; 10 were stripped 10 days later than the second lot; and 10 were stripped of all squares late in August but allowed to retain all bolls already present, after which all squares produced on this last group of plants were removed as soon as discovered. The plants in each case were selected in groups of five which were as uniform as possible in size and vigor and in the competition afforded by neighboring plants. The five treatments were then used on the plants in each group. The plants were fertilized with 500 pounds per acre of a 4–10–2 [8] fertilizer at planting and 100 pounds of sodium nitrate as a side application about July 1.

The dates of stripping and the results are given in Table 7. The variations in the square period are for the most part small and irregular. The figures do not reveal any effect from the treatment.

In the case of the boll periods, however, the results are more involved. For 1923 they agree for the two varieties in showing a progressive increase in the boll period as stripping was delayed, with the exception of the very late stripping. In this last case, however, only squares were removed, and there were only a few to remove from most plants. Shedding was abundant in all lots, so that the actual condition with this late-stripped lot was very nearly like that of the check. The value for the boll period appears to reflect this fact, as it is very nearly the same as for the check. In brief, there seems to be little in the results for this season to indicate any alteration of boll period by any of the treatments given, as the gradual increase in the period with later stripping is probably not greater than the usual increase which occurs as the season advances.

[7] It should be stated that this method of control is no longer recommended by the Florida station.
[8] See footnote 3.

TABLE 7.—*The effect of stripping the forms of cotton plants at different dates and on the duration of the square and the boll periods, 1923 and 1924*

Variety	Year and date of stripping	Blooms	Square period	Bolls	Boll period
	1923				
		Number	*Days*	*Number*	*Days*
	Unstripped	218	22.28±0.15	104	54.11±0.29
	July 12	195	21.80± .14	100	56.60± .38
	July 21	267	21.85± .14	105	56.60± .38
	July 31	229	22.40± .16	51	58.68± .49
	Aug. 25 [1]	160	21.79± .18	110	55.61± .42
Cleveland	1924				
	Unstripped	188	25.67± .18	108	66.01± .42
	July 8	191	25.50± .12	96	58.49± .48
	July 17	193	26.65± .19	49	78.12± .87
	July 27	101	26.12± .22	[2]	
	Aug. 21 [1]	151	25.22± .15	85	60.24± .53
	1923				
	Unstripped	210	21.30± .13	73	53.23± .38
	July 12	210	21.19± .13	73	54.70± .51
	July 21	232	21.02± .14	108	55.81± .44
	July 31	165	20.98± .21	31	60.16± .75
	Aug. 25 [1]	213	21.40± .12	89	54.21± .37
Webber 49–6	1924				
	Unstripped	394	26.50± .10	176	69.81± .46
	July 8	369	26.17± .09	175	65.45± .44
	July 17	344	26.28± .16	49	76.82±1.06
	July 27	265	27.13± .17	[2]	
	Aug. 21 [1]	317	25.26± .08	227	64.79± .31

[1] Squares, only, removed. All squares kept removed as fast as set during the rest of the season.
[2] No bolls matured.

The results for 1924 are more complicated. The early stripped plants had a considerably shorter boll period than the checks, which was decidedly not to be expected, whereas the ones stripped nine days later had a considerably longer one, and the ones stripped still later failed to mature any bolls. The plants from which late squares were stripped had a boll period shorter than that of the checks, and in the case of Webber shorter than that of the early stripped ones. The behavior of the early stripped plants was so unexpected that the question has been considered as to whether there were not errors in the calculations. As the original data were destroyed by fire it has not been possible to recheck them. However, all calculations were checked when made, and gross errors are not likely. Furthermore, in their general trend, erratic as they may seem, the figures for the two varieties agree with each other perfectly. If the variations in the results are due to errors of calculation it would appear that the errors had repeated themselves independently in the same order for each variety—a thing which at the worst seems very unlikely. It does not seem, therefore, that these results can be discarded as erroneous, even though the writer is unable for the present to explain them.

However, except as regards the early stripped plants, the results from the 1924 work corroborate those from the 1923 work. In general it may be said that, while not definitely proving it, the data support the proposition that stripping off the forms has very little influence on the rate of development of those which develop after the stripping.

However, it is conceivable that there was a real stimulation of development, which was masked by the longer developmental period of bolls produced in the later portions of the season. If so, it should be possible to eliminate the masking by comparing the bloom and boll periods of the forms on stripped plants with those which developed during the same part of the season on the check plants. The bloom and boll periods for the forms first observed on the check plants after the corresponding stripping dates of the other plants were therefore computed for the 1923 season. The corresponding results for 1924 could not be calculated because of the destruction of the data. The results are given in Table 8.

TABLE 8.—*Blooms, square periods, bolls, and boll periods for forms on check plants in stripping studies, 1923, first observed after the corresponding dates of stripping other plants*

[Compare with periods for stripped plants given in Table 7]

Variety	Date	Blooms	Square period	Bolls	Boll period
		Number	*Days*	*Number*	*Days*
Cleveland	July 12	196	22.24	87	54.84
	July 21	139	21.93	51	56.22
	July 31	55	22.27	7	56.30
Webber 49–6	July 12	166	21.15	47	54.30
	July 21	101	20.49	27	54.60
	July 31	13	19.08	[1] 7	[1] 54.00

[1] Includes bolls from forms first observed on July 31.

In general the square period in the Cleveland variety is slightly shorter in the stripped plants than for the corresponding intervals in the checks, but the opposite is the case in the Webber variety. The boll period is consistently greater in the stripped plants of both varieties. These data furnish no evidence that removing the forms from a plant speeds up the development of those set on later. What evidence there is—not enough to be conclusive—is in the other direction.

The flowers or fruits developed later may be of larger size and may exhibit a greater tendency to persist till ripe instead of shedding. The former point was not investigated. The bearing which the data have on the latter is shown in Table 9.

TABLE 9.—*Squares set, bolls matured, and percentage of squares maturing bolls for those portions of the season after stripping dates, and for the entire season for Cleveland and Webber 49–6 cotton, 1923*

Variety	Item	After July 12		After July 21		After July 31		Entire season	
		Unstripped	Stripped July 12	Unstripped	Stripped July 21	Unstripped	Stripped July 31	Unstripped	Stripped Aug. 25
Cleveland	Squares set—number	321	302	246	395	134	334	346	316
	Bolls matured—do	87	100	51	105	7	51	104	110
	Squares maturing bolls—per cent	27.1	33.1	20.7	26.6	5.2	15.3	30.1	34.8
Webber 49–6	Squares set—number	281	307	203	312	66	250	338	355
	Bolls matured—do	47	73	27	108	2	31	79	89
	Squares maturing bolls—per cent	16.7	23.8	13.3	34.6	3.0	12.4	23.4	25.1

This table indicates quite clearly that stripping the early forms from the plants and thus removing the competition of maturing fruits from the forms produced later permits a greater percentage of the later ones to mature. It also permits the plants to set a larger number of forms and to extend the square-production period later into the season. This last point is brought out clearly in Table 10, columns 5, 7, 9, and 10, where are shown the number of squares set on the check plants and on each group of treated plants after the date of the corresponding treatment. That the differences shown are due to chance variations in the plants is certainly very unlikely. The behavior of checks and treated plants in the early part of the season, as shown in other columns of the table, is too similar and the response after treatment too uniform to be due to such a circumstance.

TABLE 10.—*Total squares, and squares set before and following the stripping dates on check and stripped cotton plants, 1923*

Variety	Treatment	Total squares	Squares set by July 12	Squares set after July 12	Squares set by July 21	Squares set after July 21	Squares set by July 31	Squares set after July 31	Squares set after Aug. 25
Cleveland	Check	346	25	321	100	246	212	134	6
	Stripped July 12	330	28	302					4
	Stripped July 21	499			104	395			25
	Stripped July 31	565					231	334	50
	Stripped (squares only) Aug. 25 and later.	316							2
Webber 49–6	Check	338	57	281	135	203	272	66	2
	Stripped July 12	356	49	307					1
	Stripped July 21	447			135	312			1
	Stripped July 31	502					252	250	53
	Stripped (squares only) Aug. 25 and later.	355							0

The later maturity of the plants receiving the treatments was especially noticeable in 1924, when measurements of the plant heights and photographs illustrating the conditions were made at the close of the season. One of the photographs of a set of Webber 49–6 plants was published in the report of the South Carolina experiment station for 1924 (*10, fig. 4*). The plants shown here were not quite typical in that the one stripped on July 25 was taller than the one stripped on August 8, but in other respects they illustrate well the condition found at the end of the season. Unfortunately, the rest of the photographs and the measurements were among the data destroyed, and the condition can not, therefore, be presented with exactness. It can be stated, however, that the check plants and the ones from which the squares were kept removed late in the season, had ripended most of their bolls, were the shortest, and for the most part were dead. The others were progressively taller, and, both as to bolls and vegetative parts, greener as the date of stripping came later. The 10 plants of each variety stripped last were quite green and had not matured a single boll by the time the experiment was terminated by frost.

A number of previous investigators (*21; 19, p. 32–34; 32, p. 32–35; 15, p. 198; 22, p. 28*) have called attention to the fact that when the number of bolls that a plant matures is limited below what it would normally ripen the plant will respond with the production of more squares and blooms than it would produce normally. The same phenomenon is, of course, of very general observation in other plants.

Florists, in particular, know that they must remove blooms of many plants as they appear in order to get a maximum crop. Such treatment has sometimes been said to stimulate reproductive activity in the plant because the plant is thereby caused to produce more flowers than usual, and sometimes said to stimulate vegetative activity because the plant is made to continue elongation and to remain green through a longer period. Without considering the propriety of the term "stimulate" or the points involved in the two opposing points of view it might be well to call attention to the fact that in the normal cotton plant after blooming is abundant the elaborated foods go predominantly into the production of bolls and seeds and in the stripped one into the production of new vegetative growth and new forms. Unless one did not consider the maturation of bolls a reproductive activity it would seem that removing the forms must cause the subsequent development to be more vegetative in character rather than more reproductive.

One point concerning the behavior of those plants from which late squares only were removed may be worth noting. The percentage of flowers which developed into mature bolls for these plants, as shown in Table 11, was greater than for the checks in all cases except for the Cleveland plants in 1924. It does not necessarily follow from this fact, however, that removal of the late squares enables the plant to mature a larger proportion of the early squares; as the percentages in the case of the stripped plants are made higher by the removal of some squares that would have produced late blooms but could not have matured bolls. To get a reliable indication of the situation the interference of this factor on the real shedding rate must be eliminated. This has been accomplished for the 1923 data by calculating the percentage of bolls matured on the basis of the number of squares set rather than on that of blooms opened. The values are given in the last two columns of Table 9. They are in agreement with the trend exhibited by the values for the same year given in Table 11, but the differences are much smaller and might be due to chance variation. While, therefore, these results are in harmony with the idea that the competition of late set forms has a deleterious effect on the earlier ones, they can not be considered to prove it.

TABLE 11.—*Percentage of blooms which matured bolls on check cotton plants and on those plants from which late squares were removed, 1923 and 1924*

Item	Cleveland		Webber 49-6	
	1923	1924	1923	1924
Check	47.7	57.4	37.6	41.6
Plants with late squares removed	68.8	56.3	41.8	71.6

Briefly stated, stripping the forms from cotton plants early in the season can not be expected to shorten appreciably the maturation period of the squares or bolls which develop afterward, but can be expected to cause the plants to increase the number of forms set afterward and to mature a greater percentage of them, to cause the squaring period to be extended later into the season, and to cause the

plants to remain vegetatively active longer. The older bolls on a cotton plant are apparently able to appropriate a large share of the food materials which are produced during their development. As a result further development of the framework of the plant gradually comes to a stop, and young forms laid down in excess of the amount the plant can nourish to maturity are shed. In times of stress, when the amount of available water or food is diminished below normal, this shedding may assume alarming proportions. There is also a suggestion from the data that the younger forms in their turn appropriate enough of the food materials to have a slightly deleterious effect on the older bolls.

RATE OF DEVELOPMENT OF THE BOLL

Two experiments were carried out on this subject in 1923, one on the development in size and the other on the rate of hardening of the boll wall.

SIZE

The development of the bolls in size was followed by marking a large number of bolls at blossoming time and measuring the transverse diameters and lengths with calipers at regular intervals afterward. The varieties used were Cleveland and Webber 49–6 growing in the same plots in which the stripping studies were made. Unfortunately, practically all the records concerning size were destroyed, very little being left except a brief reference published in the annual report of the South Carolina Experiment Station for 1923 (*8, p. 35*) and in a circular issued early in 1924 (*9, p. 24–25*). However, it can be stated that the bolls had reached their full size in about 18 days. The time to opening can not be given closely, but it was 45 days or more. Dunnam (*18, p. 590–591*) has since reported a similar examination of boll sizes in which bolls of all stages of Dixie Triumph, Humco Cleveland, and Webber 49 were measured on a given day. The Dixie Triumph bolls were practically full sized at 17 days and the Humco Cleveland at 22 days. The Webber 49 bolls showed a rapid increase in size up to 17 days and a much slower one up to 31 days. Armstrong (*2*), reporting on Dixie Triumph at Florence, S. C., in 1926, gave a period of 20 days. Martin, Ballard, and Simpson (*30, p. 203–206*), found a mean period of 17.2 days and no increase in length of any Lone Star bolls in Texas after the twentieth day. The last-named writers found that the full size of Pima bolls in Arizona, as indicated by the volume, was reached at some time between the twentieth and twenty-fifth day from bloom, and that the full size of sea island and Meade bolls in South Carolina was reached on or about the twenty-first day. The green weights ran practically parallel with the volume; but in the case of Pima, which is the only one for which dry weights were given, the dry weight increased about 100 per cent after the bolls had reached full size by volume, reaching a maximum only at maturity. Bishara (*12, p. 8, 21*), on the basis of a rather small number of bolls measured, reported full size of Sakellarides cotton bolls on about the twenty-second day. Beckett (*11, p. 103–104*) reported that maximum length of bolls was reached in 20 days in Garo Hill, a variety of the Asiatic cotton *Gossypium cernuum*. The time required for cotton bolls to reach full size in the strains investigated up to the present is thus seen to vary from about 17 to 22 days.

Doubtless the investigation of a large number of varieties would reveal variations which could be utilized to breed strains with especially rapid boll growth, but it is rather doubtful if the result would be as valuable as an antiweevil measure as may once have appeared likely. Dunnam (*18, p. 590–593*) has shown that even full-sized bolls of upland cotton are susceptible to boll-weevil attack if the weevils have nothing else available; and the very great susceptibility of sea island bolls is well known in spite of the fact that they reach full size in about the same time required for those of upland varieties. It should be considered, though, that conditions are seldom or never as severe in the field environment as Dunnam produced artificially for his experimental bolls. Practical immunity from weevil attack under field conditions is doubtless attained much earlier than under the experimental ones. Also, it should be remembered that rapidity of development in the early stages may be associated with rapidity of development throughout the entire boll period. Ewing (*19, p. 42*) has expressed the opinion that—

> the boll reaches a stage of immunity from weevil injury before it is fully matured and the length of the period before this stage is reached apparently is more or less proportional to the total developmental period of the boll.

This seems a reasonable assumption, and if true, breeding to shorten the period of boll enlarging, if successful, should also shorten the total boll period. In that case the period of susceptibility would be decreased even though it extended almost to the ripening date.

Hardening of the Wall

The experiment on the rate of hardening of the boll wall has similarly been briefly reported, with a cut of the apparatus (*8, p. 30, 35–36*). Cleveland and Webber 49–6 varieties were used and were grown in the same plots with the plants used in the stripping studies. Four hundred blossoms of each were tagged on August 7. Ten blossoms of each variety were taken to the laboratory on the same day and 10 bolls every 5 or 6 days thereafter until maturity, and the amount of pressure necessary to make the point of a United States Bureau of Soils soil penetrometer pierce the wall was determined. The apparatus consisted of a heavy rounded-end conical needle attached to the end of a beam at the mid-point of which a bucket was hung. Sand was run into the bucket until the needle had pierced the tissues. The sand was then weighed. One-half the weight of the sand represented the pressure necessary to cause the penetration. Two readings were taken on each lock at the place of greatest transverse diameter of the boll. The value for each boll therefore represented the mean of 8 or 10 separate readings. The pressure necessary to pierce the outer layer of the wall was first taken in all cases, and then that required to pierce the inner (third) layer. This latter was considerably more in all cases. After the penetrometer readings were taken, each boll was cut across in the same plane as where punctured and the thickness of the wall of each lock measured at the thinnest place. This was done by means of parallel arm calipers calibrated to 0.05 centimeter. Readings were taken to 0.01 centimeter by estimation.

The results are shown in Table 12 and Figure 1. It will be observed that the resistance of the walls to puncture, whether the outer layer

alone or the entire wall be considered, increased rapidly at first, accompanied during this stage by an increase in thickness. The resistance by the outer layer later had ceased to increase and had bebecome practically constant some time before the bolls began to open.

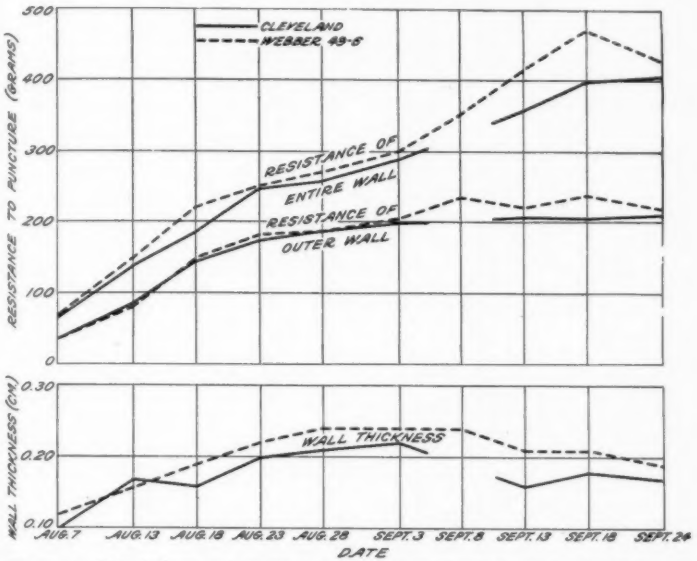

FIGURE 1.—Wall thickness and resistance to puncture of Cleveland and Webber 49-6 cotton bolls

TABLE 12.—*Resistance to puncture, and wall thickness of cotton bolls, 1923*

Variety	Item	Aug. 7	Aug. 13	Aug. 18	Aug. 23	Aug. 28	Sept. 3	Sept. 8	Sept. 13	Sept. 18	Sept. 24
Cleveland	Resistance of outer wall, grams.	38.0	86.7	144.1	175.0	186.1	196.8	------	205.5	203.8	208.3
	Resistance of entire wall, grams.	67.1	140.0	185.4	247.7	257.4	288.9	------	358.7	397.7	403.4
	Wall thickness, centimeters.	.10	.17	.16	.20	.21	.22	------	.16	.18	.17
Webber 49-6	Resistance of outer wall, grams.	37.8	81.6	148.8	181.7	186.8	203.7	234.7	219.4	236.6	217.3
	Resistance of entire wall, grams.	70.0	148.4	221.2	251.8	270.8	300.2	352.2	414.2	474.2	425.6
	Wall thickness, centimeters.	.12	.16	.19	.22	.24	.24	.24	.21	.21	.19

In the case of the total resistance, however, the increase continued at a greater rate till very near ripening time, in spite of the fact that during the latter part of the time the boll wall was actually decreasing in thickness. Finally, at maturity there was a sudden and great increase in resistance (not shown in the table or figure) due to the hardening of the boll wall as it dried out.

The walls of the Webber bolls were consistently more resistant to puncture than those of the Cleveland bolls, and the boll walls first became thicker and later somewhat thinner.

Since this work was done Armstrong (*2*) has carried out a series of similar studies on a more elaborate scale. His results agree with the writer's in that the Webber boll walls were thicker and more resistant to puncture than those of Cleveland. They apparently agree also as to boll-wall thickness. The measurements did not begin early enough to bring out clearly the initial thickening, but the later shrinkage is quite well shown by his 1925 data. The 1926 data are also consistent in a general way but with several exceptions. When it is considered that most of the thickness of the boll wall consists of more or less soft, spongy tissue it is easily understood how temporary environmental conditions influencing turgor could cause variations in thickness sufficient to mask the smaller, gradual changes.

His results, however, do not at first appear to agree with the present writer's in showing a practically continuous increase in resistance during the latter part of the boll period. As stated by Armstrong, "there was an increase in toughness of boll to about 21 days. After this time the age of the boll was not a chief factor." It is possible, though, that the failure to agree is more seeming than real and that the writer's plants were merely subjected to fewer external disturbing factors than were Armstrong's. This interpretation is given added plausibility by the fact that there are some considerable variations in the writer's values and by the added fact that Armstrong reports (*2, p. 1022*) that "some of the earlier samples were dried almost to the point of cracking open, while later samples were succulent." The writer's plants had about 6 inches of rain in August and very little in September. Water deficit can hardly have been a factor except possibly with 1 or 2 of the first and 2 or 3 of the last lots of bolls examined. It would therefore appear that the two sets of data supplement rather than contradict each other. The one points out the normal course of boll-wall hardening, and the other shows that environmental conditions can impose relatively very large variations on this course.

SUMMARY

There was little difference in the square-maturation periods of the five upland varieties of cotton considered in this study. By comparison of the periods observed with those reported by other investigators for upland and other types of cotton, it appears that the square period for this type is probably shorter than for Egyptian or for sea-island cotton.

The varietal differences in the boll periods were larger and reasonably consistent for the two years. This result is in harmony with the findings of other investigators and indicates that relative length of boll period is dependent upon hereditary characters. The breeding of strains with especially short boll periods should be feasible. Comparison of the results with those previously reported indicates that upland and Asiatic cottons have shorter boll periods than Egyptian and sea-island cottons.

Neither the amount of nitrate applied as fertilizer nor time of application had a perceptible effect on the square or boll period.

Variations in the spacing of the plants had no appreciable effect on either the square or boll period except possibly a slight shortening of the boll period in wide-spaced plants which was masked by the result of other influences. The earliness of the crop from close-spaced plants is due to other factors, and not to a shortening of the boll period.

The usual cultivation period and a lengthened period produced an increase in square and boll periods over no cultivation. This may have been due to a genuine lengthening of the period or to an apparent lengthening caused by increasing the number of bolls which matured late in the season.

Stripping forms from the plants had no perceptible effect on the square period. On the boll period, however, there was an apparent lengthening as the date of stripping was delayed. When the effect of time of the season was eliminated there was still no evidence of a shortening of the development period of forms produced on stripped plants. Stripped plants grew taller, produced more late blooms and bolls, matured a greater percentage of the late set bolls, and remained green longer in the autumn than unstripped plants.

Cleveland and Webber 49–6 bolls, which required 45 days or more to open, reached their full dimensions in about 18 days.

The resistance to puncture of the boll walls of the same two varieties increased from the time of blooming almost to maturity. At the opening of the boll there was a very great increase due to the drying out and hardening of the boll wall. The wall increased in thickness for a time and then became thinner, even while the resistance to puncture continued to increase.

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Contents

Introduction xi